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# NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

## RELATED APPLICATIONS

This application claims priority to U.S. provisional application Serial No. 60/171,329,  
5 filed December 21, 1999, which is incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides encoded therefrom.  
More specifically, the invention relates to nucleic acids encoding novel polypeptides, as well  
as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids  
10 and polypeptides.

## SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of novel nucleic acid sequences  
encoding novel polypeptides. Nucleic acids encoding the polypeptides disclosed in the  
invention, and derivatives and fragments thereof, will hereinafter be collectively designated  
15 as "FCTR" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated FCTR nucleic acid molecule  
encoding a FCTR polypeptide that includes a nucleic acid sequence that has identity to the  
nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.  
In some embodiments, the FCTR nucleic acid molecule can hybridize under stringent  
20 conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes  
a protein-coding sequence of a FCTR nucleic acid sequence. The invention also includes  
an isolated nucleic acid that encodes a FCTR polypeptide, or a fragment, homolog, analog  
or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80%  
identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:2, 4, 6, 8,  
25 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. The nucleic acid can be, for example, a genomic  
DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ  
ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a FCTR<sub>X</sub> nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29) or a complement of said oligonucleotide.

Also included in the invention are substantially purified FCTR<sub>X</sub> polypeptides (SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30). In some embodiments, the FCTR<sub>X</sub> polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a huma FCTR<sub>X</sub> polypeptide.

The invention also features antibodies that immunoselectively-binds to FCTR<sub>X</sub> polypeptides.

In another aspect, the invention includes pharmaceutical compositions which include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a FCTR<sub>X</sub> nucleic acid, a FCTR<sub>X</sub> polypeptide, or an antibody specific for a FCTR<sub>X</sub> polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a FCTR<sub>X</sub> nucleic acid, under conditions allowing for expression of the FCTR<sub>X</sub> polypeptide encoded by the DNA. If desired, the FCTR<sub>X</sub> polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a FCTR<sub>X</sub> polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the FCTR<sub>X</sub> polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a FCTR<sub>X</sub>.

Also included in the invention is a method of detecting the presence of a FCTR<sub>X</sub> nucleic acid molecule in a sample by contacting the sample with a FCTR<sub>X</sub> nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a FCTR<sub>X</sub> nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a FCTR<sub>X</sub> polypeptide by contacting a cell sample that includes the FCTR<sub>X</sub> polypeptide with a compound that binds to the FCTR<sub>X</sub> polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic

acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. The Therapeutic can be, *e.g.*, a FCTR<sub>X</sub> nucleic acid, a FCTR<sub>X</sub> polypeptide, or a FCTR<sub>X</sub>-specific antibody, or biologically-active derivatives or fragments thereof.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. The method includes contacting a test compound with a FCTR<sub>X</sub> polypeptide and determining if the test compound binds to said FCTR<sub>X</sub> polypeptide. Binding of the test compound to the FCTR<sub>X</sub> polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a FCTR<sub>X</sub> nucleic acid. Expression or activity of FCTR<sub>X</sub> polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses FCTR<sub>X</sub> polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of FCTR<sub>X</sub> polypeptide in both the test animal and the control animal is compared. A change in the activity of FCTR<sub>X</sub> polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a FCTR<sub>X</sub> polypeptide, a FCTR<sub>X</sub> nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the FCTR<sub>X</sub> polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the FCTR<sub>X</sub> polypeptide present in a control sample. An alteration in the level of the FCTR<sub>X</sub> polypeptide in the test sample as compared to the control sample indicates the presence of or

predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers.

5 In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a FCTR<sub>X</sub> polypeptide, a FCTR<sub>X</sub> nucleic acid, or a FCTR<sub>X</sub>-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, cancer, neurodegenerative  
10 disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identify the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system,  
15 affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are  
20 described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following  
25 detailed description and claims.

## DETAILED DESCRIPTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as FCTR1, FCTR2, FCTR3, FCTR4, FCTR5, FCTR6, FCTR7,  
30 FCTR8, FCTR9, FCTR10, FCTR11, FCTR12, FCTR13, and FCTR14. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "FCTR<sub>X</sub>".



The novel FCTR<sub>X</sub> nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 11A, 12A, 13A, and 14A, inclusive ("Tables 1A - 14A"), or a fragment thereof. The invention also includes a mutant or variant FCTR<sub>X</sub> nucleic acid, any of whose bases may be changed from the corresponding base shown in Tables 1A - 14A while still encoding a protein that maintains the activities and physiological functions of the FCTR<sub>X</sub> protein fragment, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including complementary nucleic acid fragments. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to 20% or more of the bases may be so changed.

The novel FCTR<sub>X</sub> proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, 11B, 12B, 13B, and 14B, inclusive ("Tables 1B - 14B"). The invention also includes a FCTR<sub>X</sub> mutant or variant protein, any of whose residues may be changed from the corresponding residue shown in Tables 1B - 14B while still encoding a protein that maintains its native activities and physiological functions, or a functional fragment thereof. In the mutant or variant FCTR<sub>X</sub> protein, up to 20% or more of the residues may be so changed. The invention further encompasses antibodies and antibody fragments, such as F<sub>ab</sub> or (F<sub>ab</sub>)<sub>2</sub>, that bind immunospecifically to any of the FCTR<sub>X</sub> proteins of the invention.

#### **FCTR1 (AL031943\_A)**

The novel FCTR1 nucleic acid encoding a C-terminal fragment of a novel FCTR1 protein is shown in Table 1A. A "TAA" stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The stop codon is shown in bold letters. This sequence originates in chromosome 6. No ATG start codon was found, indicating that the cDNA extends 5' of the disclosed sequence in Table 1A.

**Table 1A.** FCTR1 (AL031943\_A) nucleotide fragment (SEQ ID NO:1).

acccatctttttctcttctctgctgctcctaaacttaggctaccaagctttgctggggaaagcactcca  
 ggtgggtgttactacaaatcacgctctgctgacctggtactacctgacagcctttgatatttcca  
 gagtcaataacctgctttccattctccacagcatctaataagtcattggtctctcatctgtcctgctt  
 5 ccccgcttcgcgttcaccactgtgctgagatatagggaaaggaatgggaacaaggaagccatcgccgg  
 cctctccagctctggaggcttcacagcttgctcctcctcctcgtctgttgagtcacccacacgcaacc  
 acaactatgtgggagattctgtgccaggctttggcaactaa

The encoded C-terminal fragment of the encoded protein is presented using the one-  
 10 letter code in Table 1B. The protein including the C-terminal fragment disclosed has a high  
 probability of being secreted extracellularly. A signal peptide most likely is cleaved between  
 residues 19 and 20, *i.e.*, at the dash in the amino acid sequence LLG-KAL.

**Table 1B.** C-terminal fragment of the encoded FCTR1 protein sequence (SEQ ID NO:2).

15 THLFLFFVLLNLGYQALLGKALQGVTTNHRLLTHWYYLTAFDISRVNTCFPFSTASNISHGFSSVLL  
 PRFAFTTVLRYSRERNGNKEAIALSSSGGFTACLLRLLSHPTRNHNHYVGDSVPGFGN

In a search of sequence databases, no similarities were found to any known expressed  
 nucleic acid or protein. The human genomic fragment HS223B1, from clone RP1-223B1 on  
 chromosome 6p24.1-25.3, aligned with the FCTR1 nucleotide sequence, as shown in Table  
 20 1C. Putative intron and exon information can be construed from this alignment.

**Table 1C.** BLASTN alignments of FCTR1 (SEQ ID NO:1) with genomic clone HS223B1

Alignment between:

HS223B1 Human DNA sequence from clone RP1-223B1 on chromosome 6p24.1-25.3 Contains  
 STSs and GSSs, complete sequence. 5/2000 and (Pasted\_No.:1-228)

Length = 126281

Score = 452.0, bits (228.0), Expect = 1e-125

Identities = 228/228 (100%)

Strand = Plus / Plus

30 Query: 1 acccatctttttctcttctctgctgctcctaaacttaggctaccaagctttgctggggaaa 60  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 Sbjct: 1483 acccatctttttctcttctctgctgctcctaaacttaggctaccaagctttgctggggaaa 1542  
 35 Query: 61 gcactccagggtgggtgttactacaaatcacgctctgctgacctggtactacctgaca 120  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 Sbjct: 1543 gcactccagggtgggtgttactacaaatcacgctctgctgacctggtactacctgaca 1602  
 40 Query: 121 gcctttgatatttccagagtcaataacctgctttccattctccacagcatctaataaagt 180  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 Sbjct: 1603 gcctttgatatttccagagtcaataacctgctttccattctccacagcatctaataaagt 1662  
 45 Query: 181 catggcttctcatctgtcctgcttccccgcttcgcgttcaccactgtg 228  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 Sbjct: 1663 catggcttctcatctgtcctgcttccccgcttcgcgttcaccactgtg 1710 (SEQ ID  
 NO:34)

Alignment between:

HS223B1 Human DNA sequence from clone RP1-223B1 on chromosome 6p24.1-25.3 Contains  
 STSs and GSSs, complete sequence. 5/2000 and (Pasted\_No.:226-381)

Length = 126281

Score = 309.0, bits (156.0), Expect = 5e-82

Identities = 156/156 (100%)

Strand = Plus / Plus

Query: 226      gtgctgagatatagggaaaggaatgggaacaaggaagccatcgccggcctctccagctct 285  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 5 Sbjct: 4527      gtgctgagatatagggaaaggaatgggaacaaggaagccatcgccggcctctccagctct 4586

Query: 286      ggaggcttcacagcttgccctcctcctcgtctgttgagtcacccacacgcaaccacaac 345  
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
 Sbjct: 4587      ggaggcttcacagcttgccctcctcctcgtctgttgagtcacccacacgcaaccacaac 4646

10 Query: 346      tatgtgggagattctgtgccaggctttggcaactaa 381  
 ||||||||||||||||||||||||||||||||||||  
 Sbjct: 4647      tatgtgggagattctgtgccaggctttggcaactaa 4682 (SEQ ID NO:35)

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR2 (AL078594\_A)

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 2A. The initiation codon is at the 5' end, and a "TAG" stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold letters. This sequence originates in chromosome 6, in clone RP1-293L8 at map location q22.2-22.33. Homology of 100% was shown to the human genomic clone HSDJ293L8 obtained from this region, which contains the HEY2 gene for hairy/enhancer-of-split related with YRPW motif 2 (cardiovascular basic helix-loop-helix factor 1, CHF1), ESTs, STSs, GSSs and four putative CpG islands. FCTR2 nucleotide regions 1-213, 214-367, and 366-570 correspond 100% to HSDJ293L8 regions 49502-49714, 52745-52898, and 54432-54636, respectively.

**Table 2A.** Nucleotide sequence (SEQ ID NO:3) of FCTR2 (AL078594\_A).

atgactgtcaaggctcctaaagggtcataaagggtgacataacttctatactggttagttcaaacacttgc  
 tcagagctgccatgctgtgaggaggcccaagctagtcagctcagagagagcatctggagaggctctga  
 agctacacaactatagagtcctcagctgcacaagccccctgctgttccagctccaaccactgctagac  
 tacaaccatatgatactgagtaacttagccccagacgctcagggtgccactgagtatgcagtatgctga  
 cttaatcataaaaaattaacacctttagtattcaagcagctcatatcactcacaatttctctttaaca  
 aagaaaggcatgcatttcatacacggggacaattcggtcagattgtttcttcccaataacctctatgag  
 atcaattgcactgaaggaatgcctatttttactagaagaacgaagggtggaagtcaataattttgaagc  
 atggggtagcttcagaggaggagaggttcggggatcgggtacaagacttggttggggcaggataaaa  
 atactcagtatgaaaaacctgagtag

The encoded FCTR2 polypeptide sequence (SEQ ID NO.:4) is presented using the one-letter code in Table 2B. The protein appears not to have a strong probability of secretion. No signal peptide is predicted for this protein. No significant matches were found in a BLASTP search against the FCTR2 polypeptide.

**Table 2B.** Encoded FC2R2 protein sequence (SEQ ID NO:4).

MTVKAPKGHKGDITSILLVQTLAQSCHAVRRPKLVSSERASGEALKLHNYRVLSC2TSPLLFQLQPLLD  
YNHMILSNLAPDVRVPLSMQYADLI IKINTFS IQAAH ITHKFLFNKERHAFHTRGQFGQIVSSQYLYE  
INCTEGMPIFTRRTKVEVNNFEAWGSFRGGEVRGSGTRLGLGQDKNTQYEKPE

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

**FC2R3 (AL078595\_A)**

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 3A. The initiation codon is at the 5' end and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold letters. This sequence originates in clone RP3-399J4 on chromosome 6q15-16.3. No significant matches were found in a BLASTN search against the FC2R3 nucleotide sequence.

**Table 3A.** Nucleotide sequence (SEQ ID NO:5) of FC2R3 (AL078595\_A).

atgccgccactgctggctcctgctcttgctcctgccgccaccacttgccacctccctcttcagccagtg  
tggtggcagcggctgctcccgacagcccaccattcccatcagtaatatggaggggcaaatatgtgtaa  
agccttcaggtgccaaagctgctccagaaccctggaagaattatcaaagatgcgggtccctctcttca  
attccatggtatattttgtccttcagttctgcagagcctgcaatcaaacatgctaaagcagagaaata  
caataagagacctatacttgacattagcagaggaagtccagctgtgtacactaattatgataaacatc  
cattcacaatgtctgggaggagactagccacagacctggaaagaggtgaagaaaaacgacaccatgaa  
aaaggagcaaagtga

The encoded protein is presented using the one-letter code in Table 3B. The protein has a high probability of extracellular secretion. A signal peptide is predicted for this protein with a cleavage site between residues 16 and 17, *i.e.*, at the dash in the amino acid sequence PLA-PPL. No significant matches were found in a BLASTP search against the FC2R3 polypeptide.

**Table 3B.** Encoded FC2R3 protein sequence (SEQ ID NO:6).

MPPLLVLVLLLLPPLAPPLFSQCGSGCSRQPTIPISNMEGQICVKPSGAKAAPEPLEELSKMRSLSS  
IPWYILSFSSAEPAIKHAKAEKYNKRPILDISRGSPAVYTNYDKHPFTMSGRRLATDLERGEKRRHE  
KGAK

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

**FCTR4 (AL109627\_A)**

The novel nucleic acid encoding a novel transforming immortalized mammary oncogene-like protein is shown in Table 4A. An initiation codon is shown at the beginning of the sequence and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in chromosome 1 from clone RP4-733M16 at map location p36.11-36.23.

**Table 4A. Nucleotide sequence (SEQ ID NO:7) of FCTR4 (AL109627\_A).**

atggccagacctcccggtgcccggttcgggtggttggtcccaaactggcagagagtgccgaggggaagga  
 gtacctggcttgcatctctgcgcaagaaccgcccggggtgtttgggctgcttgagcggccagtgtctgc  
 10 tgcgcctgtgtccattgacactgccagctacaagatctttgtgtccgggaagagtggtgtgggcaag  
 acggcgctggtggccaagctggctggcctggaggtgcctgtggtgcaccacgagaccaccggcatcca  
 gaccacgtggtatcttggccagccaagctgcaggccagcagcgtgtcgatcatgtttcggtttgagt  
 tctgggactgtggagagtctgcactcaaaaagtctgatcatatgctgctggcttgcatggagaacaca  
 gatgccttctctctctctctctctctcactgacgtgcctccttgaagacctccctggacagctggc  
 15 ccgcatagcaggtgaggccctggtgtcgatcaggatgggtcatcggtccaaatttgaccagtacatgc  
 acacggacgtgcccagcgggacctcacagccttccggcaggcctgggagctgcccctgctacgggtg  
 aagagtgtgcccggggcgggcgtggctgatgggcgccactggacggggcggtgggtggccgacgt  
 tgccacatactcaatggccttgctgagcagctgtggcaccaggaccagacggcgatgacgccaccga  
 caggacgacgactgtgtctcgccctgcccggcatttatgtgccggactctaggggtacattttct  
 20 gagacgggaaaacctgcattgataaaagtgggacagagcgggtcagaccgctcctaactgtccccct  
 gaccccgcgatgggttagacttctgtctcgccctgggaggagaagctgcgaccccccgggcggggag  
 agaggcgactccggcagcggcgtggcgcgagaattttcagcggaaacctggaggagggcctcttgaa  
 ctgcctgggtaccaggtaccgggttcagatctcaactcttgccaattgctgtacctatactgggcttg  
 ctggggatactggcacaagtaccagccctggaccagcctttggacaaactgagctgcctcttgacc  
 25 accaggaacctgttcttcagcatcttcagctccttctggggccatggccttccctggagcactggaa  
 gcaggagtgccaccttgcccaccactgggactgcagtgacttccaggaccaggaggcaatgccag  
 ttcagccccccaccactgggactgcagcgacttccaggaccaggagggtgatgccagttcagccctcc  
 accactgggactgcagcgacttccaggaccaggaggagtgccacatctacagtttgctgccctggcc  
 ctgcagatgaccagaacctgacaggttgaaggagccctacttccaaccgcacagctgccttctc  
 30 ccactactcaccagctctgcagccatcctcactgtgctctgtgtggtgatgattttcctggtatctg  
 tcataatttaccatggcatcatcagcattgcaatgttccacactggcaactctgtgctcatgaccaa  
 gcgaatgtcctttggggcaatggaggccccaaagccctgagtaagggtgctctgtgtctgccaacaaca  
 gtgcggtcctggtggctgccacattcaggtcaccacagcagctcatcatcatcatggtgggcaaacagc  
 tgctcaaccacatggaagaatttgttgggctgggaggtggccccgggctgacactccctgcctgcc  
 35 gagctgcagtttgggttcatcaccatcttctgtgggagccttctgctggcaccctgttactctgct  
 caacaaccgggtagagattggactggacgcccacaagtctctgtgcaagtaccagcgaccaatggctg  
 ggcgcggttgacatctggatctgactgctcctgctggaggccatgtgagctgattctgccccggaca  
 aatgcgcgagccggctagggtactggctgaacgggcaggggccagattctaggggagaaggaggagg  
 aatgcggggttcggagtcgagatccgagagcctctccagacccccgaaccagatacaaggcctctc  
 40 gcgacgtgggggtgaacctcgccctcttctactggaagctgctggctgtgcatgtgcatctgggttct  
 attatcgcttcgaggggttgatgaatcaaacctcttctgtctgggtgggatctccccagccagctggg  
 cagagagaggggttcccctgcccgaacagccaaacagcatcagcagcgggctgggcccagagagggc  
 caggtgggtggcagagcaaaagaggaatggactgtgggcccactgctaccctccagccccacctgact  
 gggccacctggcactgcccaccacctgtagcagtggtgcccagcaggagagctgtcctttgcagagct  
 45 gccgcctgaagcccccgagcccagtggtctggaccttttccctgttgcccagagagctctcggg  
 ctctgggcagcgcgtgggtccctggggacccctctccaaagggttgctatggccattatcccca  
 ggaggtcagatacagagatcaccagcgggggggatgcccggcagcagggtggcagctggccacactg  
 tcctgggtggcagccccagctctggaggggaccctggagtccccgacacacacagccacagcgccggg  
 ccagccacgggtcgagagaagaagtctgcctggcgcaagatgcccgtgtaccagcgtgaagaggtcccc  
 50 ggctgccccgaggcccacgctgtcttctagagcctggccaggtagtgcaagagcaggccctgagcac

agaggagcccaggggtggagtgtgtctgggtccacccgagtgagcctcgaaggtcctgagcggaggcgct  
tctcggcatcggagctgatgacccggctgactcttctctgcgctggggcggaattcagcagcccgg  
gcactcatctctgggtcaggcaccggagcagcccgggaagggaagcatctggaatggaggctcgaag  
5 tgtagagatgagcggggaccgggtgtcggggccagcccctgggtgactcacgagagggcgatttggtccg  
agcccaggctagacacacaggaagagccgcctttgggggtccaggagcaccaacgagcggcgccagtct  
cgattcctccttaactccgtcctctatcaggaatacagcgacgtggccagcgcccgcgaactgcggcg  
gcagcagcgcgaggaggaggggccgggggacgaggccgagggcgcagaggagggggccggggccgcccgc  
gggccaacctctccccagcagctccttccgggcgagcgctcggcgcgaggctccaccttctcgctg  
10 tggcaggatatccccgacgtacgcggcagcggcgctcctggccacgctgagcctgcgggactgcaagct  
gcaggaggccaagtttgagctgatcacctccgaggcctcctacatccacagcctgtcggtggctgtgg  
gccacttcttaggctctgccgagctgagcgagtgctctgggggagcaggacaagcagtggtgttttcc  
aaactgcccagggtcaagagcaccagcgagaggttctcgcaggacctggagcagcggctggaggcaga  
tgtgtcgcttcagcgtgtgcgacgtgggtgctggaccactgccggccttccgcagagctacctgc  
cctatgtcaccaaccaggcctaccaggagcgacctaaccagcgctgctcctggagaacccaggttc  
15 cctggcatcctggctcgctggaggagtccctgtgtgtgagcgtctgccccttacctccttcttctat  
cctgcccctccagaggatcacccgcctcaagatgttggtggagaacatcctgaagcggacagcacagg  
gctctgaagacgaagacatggccaccaaggccttcaatgcgctcaaggagctggtgcaggagtgaat  
gctagtgtacagtcctgaagaggacagaggaactcatccacctgagcaagaagatccactttgaggg  
caagattttcccgctgatctctcaggcccgcgtggctggttcggcatggagagttggttagagctggc  
20 cactgcctgcagcacccttgcgaagctgaagctgtccagcaaggcagctctacctccacctcttcaat  
gactgcttgctgctctctcggcggaaggagctagggaagtttgccgttttctgctccatgccaagatggc  
tgagctgcaggtgcgggacctgagcctgaagctgcagggcacccccggccacgtgttctcctccagc  
tctccacgggcagcacatgaagcaccagttcctgctgcggggccggacggaaagtgagaagcagcga  
25 tggatctcagccttggtgcccctccagccccagaggacaaggaggtcatcagtgagggggaagattg  
ccccagggttcagtggttaggacatacaaggcactgcaccagatgagctgacctggagaagactg  
acatcctgtcagtgaggacctggaccagtgcggctggctggaaggggtccgcctggcagatggtgag  
aaggggtgggtgccccaggcctatgtggaagagatcagcagcctcagcgcccgcctccgaaacctccg  
ggagaataagcgagtcacaagtgccaccagcaaaactgggggagggtcctgtgtga

30 The encoded protein is presented using the one-letter code in Table 4B. The protein has a high probability of sorting into the plasma membrane. No signal peptide is predicted to occur for this protein.

**Table 4B.** Encoded FCTR4 protein sequence (SEQ ID NO:8).

MARPPVPGSVVVPNWHESAEGKEYLACILRKNRRRVFGLLERPVLLPPVSIDTASYKIFVSGKSGVGK  
35 TALVAKLAGLEV PVVHHETTGIQTTVVFWPAKLQASSRVVMFRFEFWDCGESALKKFDHMLLACMENT  
DAFLFLFSFTDRASFEDLPGQLARIAGEAPGVVRMVGSKFDQYMHTDVPERDLTAFRQAWELPLLRV  
KSVPGRRRLADGRTLDRAGLADVAHILNGLAEQLWHQDQTAMTPPTGRRLCLAPCGGIYVPDSRGTF  
ETGKPALIKVGQSGVRPLLTVP LTPRWVRLRLRLGGEAATPAAAGERRLRQRRWRENFRQNL E E GLFE  
40 LPGYQVPGSDLNSCQLLYPYWACWGYWHKYQPLDQPLDKLSCLFDHPGTVFFSIFMSFWGHGLPGALE  
AGSATLAHHWDCSDFQDQEAMPSSAPHHWDCSDFQDQEVMPSSALHHWDCSDFQDQEECPHLQFAALA  
LQMTQNPTVTLKEPYFQPHSCLSHLLTSSAAILTVLCVVMIFLVSVI IYHGIISIAMFHTGNSVLM TQ  
ANVLWGNGGPKALS KVLVCVQQCGPGGCHI QVTQQLIIIMVGKQLLNHMEEFVGLGGGPGPDT PCLP  
45 ELQFGFITIIFVGAFL LAPLFTLLNNRVEIGLDAHKFLCKYQRP MAGRGWTS GSDCSCWRPCELILPRT  
NARSRLGYWLNQGGQILGRRRGGNAGFGVEIREPLQTPQPRYKASRDVGVNLALFYWKLLAVHVLG F  
I IAF E GLMNQTLCLGGISPSQLGRERAS PAGTAKQHQQRAWAQRGPGGWQSKRGMDCGPPATLQPHLT  
GPPGTAHPVAVCQQESLSFAELPALKPPSPVCLDLFPVAPEELRAPGSRWSLGTAPLQGLLWPLSP  
GGSDTEITSGMRPSRAGSWPHCPGAQPPALEGPWSPRHTQPQRASHGSEKKSAWRKMRVYQREEVP  
50 GCPEAHAVFLEPGQVVQEALSTEEPRVELSGSTRVSLGEPERRRFSASELMTRLHSSLRLGRNSAAR  
ALISGSGTGAAREGKASGMEARSVEMSGDRVSRPAPGDSREGDWSEPRLD TQEEPLGSRSTNERRQS  
RFLLSNVLYQEYSDVASARELRRQREEEGPGDEAEGAEEGPGPPRANLSPSSSFRAQRSARGSTFSL  
WQDIPDVRGSGVLATLSLRDCKLQEAKFELITSEASYIHSLSVAVGHFLGSAELSECLGAQDKQWLFS  
KLPEVKSTSERFLQDLEQRLEADVLRFSVCDVLDHCPAFRRVYLPYVTNQAYQERTYQRLLENPRF

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**Table 4C.** BLASTN identity search of FCTR4 and the hGEF cDNA (SEQ ID NO: 36).

Score = 1856 (278.5 bits), Expect = 7.2e-78, P = 7.2e-78  
Identities = 1120/1772 (63%), Positives = 1120/1772 (63%), Strand = Plus / Plus

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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52

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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	5
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x=0;
for i=1:10
    x=x+1;
end

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[illegible]

Query: 5500 GGAGGGCCCCGGGGGACGAGGCCCGA C GGGCCAG AGGAGGGGGGGGGG GGGGGGGGGG 5494

Query: 3435 GCCAACCCCTCTCCCCAGCAGCTCTCTCTGGGCGCAGCGCTCGGCGCGAGGGCTCCACCTTC 3494

Query: 3495 TCGCTGTGGCAGGAATATCCCCGACGTAACGCGGCAGCGGCCTCCCGCCACGCTG-AGCCCT 3553

Query: 3334 GCGG-GAC1GCAAGC1GCAGGAGGCCAAG111GAGC1GATCACC1CCGAGGCC1CC1ACA 3612

query: 3613 TCCACAGCCCTGTCGGTGGCT-GTGGGCCACCTCTTAG-GCTCTGCCGAGCTGAGCGAGTG 3670

Query: 3671 TCIGGGGGCGCAGGACAAGCAGTGGCTGTCTTCCAAACCGCCCGAGGTCAAGAGCAC-CA 3729

09746491-071301

Sbjct: 999 CCTGCACCCGTCCGAGGCGCACATCCTCTCTCCAACGTCCTGGACGTGCTG-GCTGTCA 1057  
 5 Query: 3730 GCGAGAGGTTCTCTGCAGGACCTGGAGCAGCGGCTGGAGGCAGATG-TGCTGCGCTTACG- 3787  
 Sbjct: 1058 GTGAGCGGTTGGTCTCTGGAGCTGGAGCACCGGATGGAGG-AGAACATGGTCATCT-CTGA 1115  
 10 Query: 3788 CGTGTGCGACGTGGTGTCTGGACCACTGCCCGGCTTCCGCAGA--GTCTACCTGCCCTA 3844  
 Sbjct: 1116 CGTGTGTGACATCGTG-T--ACCGTTATGCGGCCGACCACTTCTCTGTCTACATCACCTA 1172  
 15 Query: 3845 TGTCACCAACCAGGCCTACCAGGAGCGCACCTACCAGCGCCTGCTCCTGGAGAACCCAG 3904  
 Sbjct: 1173 CGTCAGCAATCAGACCTACCAGGAGCGGACCTATAAGCAGCTGCTCCAGGAGAAGGC-AG 1231  
 20 Query: 3905 GTTCCCTGGCA-TCCTGGCTCGCCTGGAGGA-GTCTCCTGTGTGCCAGCGTCTGCCCTT 3962  
 Sbjct: 1232 CTTTCCGGGAGCTGATCGCGCAGCTAGAGCTCGACCCAA-GTGCAGGGGGCTGCCCTT 1290  
 25 Query: 3963 ACCTCCTTCCTTATCCTGCCCTTCCAGAGGATCACCCGCCTCAAGATGTTGGTGGAGAAC 4022  
 Sbjct: 1291 TCCTCCTTCCTCATCTGCCTTTCAGAGGATCACACGCCTCAAGCTGTTGGTCCAGAAC 1350  
 30 Query: 4023 ATCCTGAAGCGGACAGCACAGGGCTCTGAAGACGAAGA-CATGGCCACCAAGGCCTTCAA 4081  
 Sbjct: 1351 ATCCTGAAGAGGGTAGAAGAGAGGTCTGA-GCGGGAGTGCACTGCTTTGGATGCTCACA 1409  
 35 Query: 4082 TGCGCTCAAGGAGCTGGTGCAGGAGTGCAATGCTAGTGACAGTCCA-TGAAGAGGACAG 4140  
 Sbjct: 1410 GGAGCTGGAAATGGTGGTGAAGGCATGCAACGAGGGCGT-CAGGAAAATGAGCCGCACGG 1468  
 40 Query: 4141 AGGAACCTCATCCACCTG-AGCAAGAAGATCCACTTTGAGGGCAAGATTTTCCCGCTGATC 4199  
 Sbjct: 1469 AACAGATGATCAGCATTCAG-AAGAAGATGGAGTTCAAGATCAAGTCGGTGCCCATCATC 1527  
 45 Query: 4200 TCTCAGGCCCGCTGGCTGGTTCGGCATGGAGAGTTG--GTAGA-G-CTGGCACCCTGCC 4255  
 Sbjct: 1528 TCCCACTCCCGCTGCTGCTGAAGCAGGGTGAGCTGCAGCAGATGTGAGGCCCAAGACC 1587  
 50 Query: 4256 TGCAGCACCCCTGCCAAGCT-GAAGCTGTCCAGCAAGGCAGTCTACCTCCACCTCTTCA 4314  
 Sbjct: 1588 TCCCGGACCC--TGAGGACCAAGAAGCTCTT--CACGAAATT-TACCTCTTCTGTTC 1642  
 55 Query: 4315 ATGAC-TGCTTGCTGCTCTCTCGGCGGAAGG-AGCTAGGGAAGTTTGCCGTTTTCTGC-C 4371  
 Sbjct: 1643 ACGACCTGCTGG-TGATCTGCCGGCAGATTCCAGG-AGACAAGTACCAGGTATTTGACTC 1700  
 60 Query: 4372 ATGCCAAGATGGCTGAGCTGCAG-GTGCGGGACCTGAGCCTGAAGCTGCAGGGC-ATCCC 4429  
 Sbjct: 1701 A-GCTCCGCGGG--GA-CTGCTGCGTGTGG-AG--GAGC-TGGAGGACCAGGGCCAGACG 1752  
 65 Query: 4430 C-GGCCA-CGTGTTCTCTCCAGCTCCTCCAGGGCAGCACATGAA-GC-A--CCAGTT 4483  
 Sbjct: 1753 CTGGCCAACGTGTTTCATCTGCGGCTGTGGA-GAAC-GCAGATGACCGGGAGGCCACCT 1810  
 Query: 4484 CC-TGCTGCGGGCCCGGACGGAAGTGAGAAGCAGCGATGGATCTCAGCCTTGTGCCCT 4542  
 Sbjct: 1811 ACATGCTAAAGGCGTCTCTCAGAGTGAGATGAAGCGTTGGATGACCTCACTG-GCCCC- 1868  
 70 Query: 4543 CCAGCCCCCAGGAGGAC-AAGGAGGT--CAT-CAG-TGAGGGG-GAAG-ATTGCCCCCAG 4595  
 Sbjct: 1869 CAA---C-AGGAGACCAAGTTTGTTCGTTACATCCCGGCTGCTGGACTGCCCCCAG 1923  
 75 Query: 4596 GTTCAGTGTGTTAGGACATACAAGGCACTGCACCCAGATGAGCTGACCTTGAGAGAAGACT 4655  
 Sbjct: 1924 GTCCAGTGGCTGCACCCATACGTGGCTCAGCAGCCAGACGAGCTGACGCTGGAGCTCGCC 1983  
 Query: 4656 GACATCCTGT-CAGTGAGGACCTGGACCAGTGACGGCTGGCTGGAAGGGGTCCGCCTGGC 4714



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TIM\_HUMAN  
AL109627  
Q99434\_Human

RITRLKLLQNILKRTQPGSSEAEATKAHHALEQLLRRCNNNVQSMRRTTEELIYLSQKI  
RITRLKLLQVENVILKRTAQGSEEDMATKAFNALKELVQECNASVQSMKRTTEELIHLSKKI  
RVTRLPLLLMDTECLKIQGHSEERYKAASRLAKATSKLVRCNEGAAHMRMERMEQMYTHTQV

TIM\_HUMAN  
AL109627  
Q99434\_Human

EF-ECKIFPLISQSRWLVSQELTALE-FSSAPGLRRLKLNTR-VHLHLFNDCLLLSRPR  
HF-ECKIFPLISQARWLVRHGVLELAPLPAAPAKILKLSKA-VYLHLFNDCLLLSRRK  
DESKVSLPLISQSRWLVRHGVLELAPLPAAPAKILKLSKA-VYLHLFNDCLLLSRRK

```

TIM_HUMAN      EGSRFELVFDHAPFSSIRGEKCEMKLHGPHK-----NLEFRIFLFRONTQGAQAEFLER
AL109627      ELGKFAVVFVHAKMAEEQVRDLSKLGQIPG-----HYFLLQLLHG-QHMKHQFLLR
Q99434_Human   SEESYVVQDYAQMNHIOVEKIEPSELPLPGGGRSSSVPHFQVITLLRNSEGRQEQLLLS

5  TIM_HUMAN      RETQSEKLRWISALAMPREELD---LLECYNSPQVQCIRAYKPRENDELALEKADVVMVT
AL109627      ARTESEKQRWISALCPSPQEDKEVISEGEDCPQVQCVRITYKALHPDELTKLEKTDLSVR
Q99434_Human   SDSASDRARWIVALTHSERQWQG--LSSKGLLPQVEITKAFFAKQADEVTLEQQADVVEVL

10 TIM_HUMAN      QSSSDGWLEGVRLSDGERGWFPVQCVFISNPEVRAQNLKEAHRVKTKAKLQIVEQQA
AL109627      TWTSDGWLEGVRLADGERKGWVPQAVVEEISSLSARLRLNRENKRVTSATSKLGEAPV
Q99434_Human   QC-EDGWLVCERLDGETGWFPDEARFITSRVAVEGNVRMERLVRVETDV-----

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From these analyses, it is seen that the FCTR4 AL109627\_A nucleic acid and protein are similar to the TIM oncogene. The transforming gene, designated TIM, encoded a predicted protein species of 60 kDa containing a Dbl-Homology (DH) motif. This motif is also present in other growth regulatory molecules including Bcr, Cdc24, Vav, Ras-grf, and Ect2 which have been implicated as regulators of small GTP-binding proteins. NIH3T3 cells transfected with TIM expression plasmid showed altered growth properties in vitro and were tumorigenic when injected into nude mice. The 6.5 kilobasepair (kb) transcript of the TIM gene was found to be expressed mainly in kidney, liver, pancreas, lung, and placenta.

**Table 4F:BLAST alignment of FCTR4**

BLAST alignment file included sequences:

```

Line 2 > gi|11420361|ref|XP_004812.1| Oncogene TIM [Homo sapiens] (SEQ ID
NO:39)
Line 3 > gi|4885633|ref|NP_005426.1| Oncogene TIM [Homo sapiens] (SEQ ID
NO:40)
Line 4 > gi|9845277|ref|NP_063920.1| neuronal guanine nucleotide exchange
factor [Mus musculus] (SEQ ID NO:41)

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1030      1040      1050      1060      1070      1080
FCTR4      ....|....|....|....|....|....|....|....|....|....|....|....|
Line 2      -----EGSSDSRGPAVEKHGPGSD-----TVVFREKKPKVEVM
Line 3      -----MIHPIPADSWRNLTIEQIGTLYQEYRDKSTL
Line 4      -----MIHPIPADSWRNLTIEQIGTLYQEYRDKSTL

1090      1100      1110      1120      1130      1140
FCTR4      STNERRQSRFILNS-VLYQEYSDVASARELRRQQRBEEGPGDEAEGAEEGPGPPRANLSP
Line 2      GGFSRRCSKLINSQOLLYQEYSDVVLNKEIQSQQRLES--LSETPGPSS-PRQPRKALVS
Line 3      GGFSRRCSKLINSQOLLYQEYSDVVLNKEIQSQQRLES--LSETPGPSS-PRQPRKALVS
Line 4      QEIETR-----RQDAEIQNSDGSQVGEDAGEEEEEEEEGEELASP---PERRALPQ

1150      1160      1170      1180      1190      1200
FCTR4      SSSFRAQRSARGSTFSLWQDIPDVRGSCVLAITLSLRDCKLQEAQFELITSEASYTHSLSV
Line 2      SE-SYLQRLSMASGCSLWQETPVVRNSTVLLSMTHEDCKLQEVKFELIVSEASYLRSNLN
Line 3      SE-SYLQRLSMASGCSLWQETPVVRNSTVLLSMTHEDCKLQEVKFELIVSEASYLRSNLN
Line 4      IC---LLSNPHSRFNLWQDIPETQSSGVLDITQPEIRLQEAQFELIVSEASYLRSNLN
Cons      S   LWQ   P   S   VL           LQE   FEL   SEASY   SL

1210      1220      1230      1240      1250      1260
FCTR4      AVGHFLGSAELSECLGAQDKQWLFSKLPVVKSTSERFLQDLERLEADVLRFSVCDVVL
Line 2      AVDHFLQSTSLRATLSNQEHQWLFSRLQDVRDVSATFLSDLEENFENNIFSFQVCDVVLN
Line 3      AVDHFLQSTSLRATLSNQEHQWLFSRLQDVRDVSATFLSDLEENFENNIFSFQVCDVVLN

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Line 4 LVSHFMENRERLKKIHPSEAHILFSNLDVMAVSEFILLEHRMEENIVISDVCDIVYR

1270 1280 1290 1300 1310 1320

5 FCTR4 HCPAFRRVYLPYVTNQAYQERTYORLLLENPRFPGHLLARLEESPVQCRLPLTSFLILPFO  
 Line 2 HAPDFRRVYLPYVTNQTYQERTFOSLMNSNSNFREVLEKLESDPVCQRLSLKSFLILPFO  
 Line 3 HAPDFRRVYLPYVTNQTYQERTFOSLMNSNSNFREVLEKLESDPVCQRLSLKSFLILPFO  
 Line 4 MAADHFSVYITVSNQTYQERTYKQLLOEKAFREHQAQLELDPKCKGLPFSSFLILPFO

1330 1340 1350 1360 1370 1380

10 FCTR4 RITRLKMLVENILKRTAOGSEEDMATKAFNALKELEVOECNASVQSMKRTTEELIHLSSKKI  
 Line 2 RITRLKLLQONILKRTOPGSSBBAEATKAHHALEQLIRECANNVQSMRTEELIYLSQKI  
 Line 3 RITRLKLLQONILKRTOPGSSBBAEATKAHHALEQLIRECANNVQSMRTEELIYLSQKI  
 15 Line 4 RITRLKLLQONILKRVSEEREGETALDAHKELEMMWKAACNEGVKMSRTEOMISTOKKM

1390 1400 1410 1420 1430 1440

20 FCTR4 HFECKIFPLISQARWLVRHGELVELAPLPAPPAKLLKSSKAVYLLHLFNDCLLLSRREL  
 Line 2 EFECKIFPLISQSRWLKSGELTALEFS-ASPGLRRKLNTRPVHLHLFNDCLLLSRPREG  
 Line 3 EFECKIFPLISQSRWLKSGELTALEFS-ASPGLRRKLNTRPVHLHLFNDCLLLSRPREG  
 Line 4 EFKIKSVFIIHSRWLKKOGELOQMSPGKTSRTLRTKKLFREYLLFLFNDLLVLCROIPG

1450 1460 1470 1480 1490 1500

25 FCTR4 GKFAVFVHAKMAELQVRDLSSEKLOGIPGHVLLQLLHG-QHMKHQFLLRARTESEKORWI  
 Line 2 SRFLVFDHAPFSSIRGEKCEMKLHGPHKNLEFRLFLRONTQGAQAEFLFRITETOSEKLRIWI  
 Line 3 SRFLVFDHAPFSSIRGEKCEMKLHGPHKNLEFRLFLRONTQGAQAEFLFRITETOSEKLRIWI  
 Line 4 DKYQVFD SAPRGLLRVEELEDOGQ-TLANVEILRLLENADDEATYMLKASSOSEMKRWY

1510 1520 1530 1540 1550 1560

30 FCTR4 SALCPSSPOEDKEVISEGEDCPQVQCVRITYKALHPDELTLLEKLDLSVRTWISDGLWLEGV  
 Line 2 SALAMP---REELDLECYNSPOVQCIRAYKPRENDELALAKADVMMVTQSSDGLWLEGV  
 Line 3 SALAMP---REELDLECYNSPOVQCIRAYKPRENDELALAKADVMMVTQSSDGLWLEGV  
 35 Line 4 TSLAPNRRRTKFVSFTSRLLECPQVQCVRHPYVAQOPDELTLLEADLNMLEKTEDGWIIFGE

1570 1580 1590 1600 1610

40 FCTR4 RLADGEGWVPOAYVEEISSLSARLENLRENKRVTSATSKLGEAPV-----  
 Line 2 RLSGGERGWFPVQOVVEFISNPEVRAQNLKEAHRVKHAKLOLVEQQA-----  
 Line 3 RLSGGERGWFPVQOVVEFISNPEVRAQNLKEAHRVKHAKLOLVEQQA-----  
 Line 4 RLHDQERGWFPSSMTBEILNPKIRSQNLKECFRVHKMEDPQRSQNKDRRLGSRNRQ

45 FCTR4 was found to have high homology to the domains shown in Table 4G.

Table 4G: CD domain analysis of FCTR4

Sequences producing significant alignments:	Score (bits)	E value
Guanine nucleotide exchange factor for Rho/Rac/Cdc42-like GTPa...	110	7e-25
RhoGEF, RhoGEF domain	69.3	1e-12
ras, Ras family	61.2	4e-10
Rab subfamily of small GTPases; Rab GTPases are implicated in ...	51.2	4e-07
SH3, SH3 domain	49.7	1e-06
Rho (Ras homology) subfamily of Ras-like small GTPases; Member...	44.7	4e-05
Src homology 3 domains; Src homology 3 (SH3) domains bind to t...	43.9	6e-05
Ras subfamily of RAS small GTPases; Similar in fold and functi...	38.9	0.002
arf, ADP-ribosylation factor family	38.1	0.003

The AL109627\_A nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various cancers, tumors and similar neoplastic diseases. For example, a cDNA encoding the transforming immortalized mammary oncogene-like protein may be useful in gene therapy, and the transforming immortalized mammary oncogene-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding transforming immortalized mammary oncogene-like protein, and the transforming immortalized mammary oncogene-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

#### **FCTR5 (AL109913\_A)**

The novel FCTR5 nucleic acid encoding a C-terminal fragment of a novel FCTR5 protein is shown in Table 5A. This sequence contains no initiation codon. A TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The stop codon is shown in bold letters. This sequence originates in chromosome X, clone RP11-183K14, and is found at map location q26.3-27.3.

**Table 5A. FCTR5 (AL109913\_A) C-terminal nucleotide fragment (SEQ ID NO:9).**

```

natgatgatgagcaaacatgatttcaatattgagcctgggtgtctgtgaccattgctgtgttcatccc
agttgcctgtgacagtcacatgatcaacaagtctgcaccatgaccttctcatctccatatccagtgccca
agttattcctttccccaactgcaggccccccaacaggatgtgggcagcctgcattctccgctggactgg
agccaaatgccaaagcacagcaccttcgagttccatgcctccagaagggttgccctgcgcactgg
gatgggtgcttgtttgcaagggttatagatgagaaaactgctgccttgctcggaaggaaagggtgctgttg
gtctcttcgctggcatccccatctttaggaattccagcccaacaagccgccttccaattag

```

The encoded C-terminal fragment of the encoded protein is presented using the one-letter code in Table 5B. The C-terminal fragment disclosed has a very high probability of being secreted extracellularly. A signal peptide most likely is cleaved between residues 28 and 29, *i.e.*, at the dash in the amino acid sequence CDS-HDQ.

**Table 5B. Encoded FCTR5 polypeptide sequence (SEQ ID NO: 10).**

```

XDDEQNMISILSLVSVTIIVFIPVACDSHDQVCTMTFSSPYVPKFLSPTAGPPTGCGQPASPLDW
SQNAKAQHRLRVPCLQKGLSLRTGMVLVCKVIDEKTAALSEGKVLFGLFAGIPIFRNSSPNKPPSN

```

In a search of sequence databases, no similarities were found to any currently disclosed nucleic acid or protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### **FCTR6 (AL109928\_A)**

5 A novel nucleic acid encoding a novel transmembrane protein is shown in Table 6A. It was identified in chromosome 20 clone RP4-551D2 at map location q13.2-13.33. An initiation codon is shown at the beginning of the sequence and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. These are shown in bold face in Table 6A.

10 **Table 6A. Nucleotide sequence (SEQ ID NO:11) of FCTR6 (AL109928\_A).**

15 **at**gagatccgggagggcaccctcgcgtgctgctgcttctagtgcgtgctgctgtggctgctgcaggctcag  
tatcattgacagtgttcaacaggaacagatgatcttactaagcaacaaagtgtcactataagttcc  
aggaaaagatctaccagcctctacggcgatccaagagaagatgggttatcaccaccttggagctggag  
gaggaagaccgggaccctttcccaaactcattgggtgagctgttcaataatatgtcttataacatgtc  
20 actaatgtatctaatacagtggaacctgggtggtgaatatccagagattgggtttgttttctctagaag  
atcatgagaacgggaaggatatatgttcaccgacctgtcgatcgagaaatgacaccatctttcacgagc  
tggacagcaaggggtgccttctccagggcttccgcggggatgagcagaggccatctacgggaagggct  
gggtgctgggtttatatttgatgttgtgagcgctcaacaggaataattgtggatacatccttgattttca  
acattaggatcagtgatgtgaatgatgcacccaggttccagagaaggaatttaacatcactgtg  
25 caagaaaaccaatctgcagggcaacctatttttcagatgttagcagtcgatttggatgaagaaaacac  
tccaaattctcaagtcctttacttccctcattttctcaaaccattactgaaagaaagtgggttccggg  
ttgatcgaccttagtgagaaatacgaactctctggctgcttagattatgagaccgctcctcagtttaca  
ctgctaatacagagccagggaactgtggagaaccgtcactgtcatccacgaccaccgttcacgtggatgt  
gcaagaaggcaacaaccacaggcctgcatttaccagggagaactataagggttcagattcctgaaggcc  
30 gagccagccagggcggtgttgcgtctcctgggttcaagatcgagattctccatttacatcagcttgaga  
gcaaaattcaacatattgcatggcaatgaagaggggcattttgacatttcgaactgaccctgagaccaa  
cgaagggatattaaatgttatcaagcctttggattatgagactcgccagcgcaaaagcctcatcattg  
tcgtggagaatgaggagaggctcgtctctctgtgagagaggaaagcttcagccgccaagggaaggcagca  
gccagcgccactgtgagtggtgcaggtgacagacgccaacgaccaccagcctttcacccccagagctt  
catttgtcaataaagaggagggcgccaggcctgggaccctgttgggaacttttaatgccatggatccag  
35 acagccagataagatatgaactgggtcatgaccagcaaatgggtcagcgtcgacaaaaactccgga  
gtggtcatcacgctggagccaattgaccgagaatcccctcatgtaaataacagtttttatgtaatcat  
cattcacgctgttgatgatggcttcccaccgcagactgctacagggaacctaatgctcttctcgtctg  
acatcaatgacaacgtcccgaactctccggccagcttcccgtacatggaggtctgtgagctctgctgtg  
catgagccccctccacatcgaggcagaggatccggacctggagccgttctctgacctttacatttga  
40 attggacaataacctggggaaatgcggaggacacatggaagtggggagaaattggggaaactctcctc  
atcagggggtaggaggctgctgggagtccttgagacataattcttgcatctggcaagaagggtgtttcc  
agggaagctccaggattgacgtcactgtttggcctgggtcaatcagttgaacttttaaccttgagaag  
cctgccacgtggtaattacttgggtgccactcttcattggagacaaacagggaactttccagaaagcaaa  
ctgtccatgtaaggatctgccccgtgcccagtgggctcacatgtgtggagcttgcagatgcagaagtg  
gggttcatgtgggggcccctgttccctgtctgtgcagcatttgtggctctggcagtggtctgtcttt  
tctgttgcatgctattttgtgcttgaacctaaagaggcatggatgctctgtatccaatgatgaaggcc  
45 accaaacactgggtcatgtataatgcggagagcaaaaggcacttcagcccagacatgggtcagatgttgaa  
ggccagaggccggctctgctcatctgcacagctgcagcaggaccacgcaggaggttaaggggagggga  
accaaagcctccaccttctaggttttgggtgatctctgggttcccttcagtgctcctgcaaatattgta  
gatctcgaggaagtgctccatctgcagcagctcagtcagcccaagcacgctgtgctctggggagctg  
gatagcacagagaccagatccacagacatggggccagatgagcaggagactgccagcagcccatcatg

ggaaacaatgggcagccctgcagaatgggtgctgctggcacctgcttcaagacaacacagacatctt  
 ctccgggcctagaagctttgcctaaaagcagggaagccaggctcctgcagaagggggtgtgtacca  
 cagactcagggtgcagggcccttccccaggctcctgactgctgaactggaaatgggggtggaggacag  
 agaaagaacagaggctcttggggaggctttcatggccaggctggctgccgacctgaagggggactatc  
 5 tgcagagcttgggaagggaggcatccacagtgggaatcctgtgttgggaaggagccagagtccctcacac  
 tggcaggccaaaaaggcctggatccccaaacttttcaaaaagagaaataaattcaacaacgtagcacc  
 tatagtcaacaacgtagcatctatagtcaacaacatagcacctatagtcaacaacgtagcacctatag  
 tcaacaacgtagcatctatagtcaacaacgtagcacctatagtcaacaacgtagcacctatagtcaac  
 aacatagcacctatagtcaacaacgtagcatctatagtcaacaatgcacttcaacattttactttaag  
 10 tgctaggatacatgtgcagaaggtgcagtctaaagagagaaatcgcttcagcctcagcagggggtgca  
 tcatccccagggaagagccacagctgggcgaggattgccacaagacatttacaaggagatgatgccca  
 cggagactaacgcagactggtaaacggaaacacggggccttggctcgaacaccctcttcaagaaagt  
 tgtttatgaccacaaggaagtgtctctcatctgttgggtacaaacatccccagaagatccccgccac  
 acattccctggatcagaacccatcagtggttccctagtgctgctgggaatttccattcaatggcctccga  
 15 accatgagcctgccttttctgctgaagcccaaacccagctacagatctttaccccagagaccatc  
 ttgggcctccctccaggcttttgccttactctgtgcccctcatcctggagtctgtccccaccctatct  
 acagaaactccaccagcctcctggctgccccgatggctcctcgacagggagacttgtctacctcccg  
 aggtcacgtgtgggctctggctcctcttgcctcatggcagagatcttctgtatctccccctggctgc  
 20 tgggtgctctgcttacctcctccagagttgttaacaaagagctgaggatgctgagctgccagggactt  
 ggctgcaggtggcatag

The encoded protein is presented using the one-letter code in Table 6B. The protein  
 has a high probability of sorting into the plasma membrane. Cleavage of a signal peptide is  
 predicted to occur between residues 27 and 28, *i.e.*, at the dash in the sequence IDS-VQQ.

**Table 6B.** Encoded FCTR6 protein sequence (SEQ ID NO:12).

MRSGRHPSLLLLLVLLLWLLQVSIIDS VQQETDDLTKQTKCHYKFQEKIYQPLRRSKRRWVITTTLELE  
 EEDPGPFPKLIGELFNMSYNMSLMYLSISGPGVDEYPEIGLFSLEDHENGRIYVHRPVDREMPSTFTS  
 WtarVPSSRASAGMSRGHLREGLVLVYFDVVERSTGKIVDTSLIFNIRISDVNDHAPQFPEKEFNITV  
 30 QENQSAGQPIFQMLAVDLDEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEIRLSGCLDYETAPQFT  
 LLIRARDCEPSLSSTTVHVDVQEGNNHRPAFTQENYKVQIPEGRASQGVRLLLVQDRDSPFTSAWR  
 AKFNILHGNEEGHFDISTDPETNEGILNVIKPLDYETRAQSLIIVVENEERLVFCERGLQPPRKAA  
 ASATVSVQVTDANDPPAFHPQSFIVNKEEGARPGTLLGTFNAMDPDSQIRYELVHDPANWVSVDKNSG  
 VVITVEPIDRESPHVNNSFYVIIHAVDDGFPPTATGTLMLFLSDINDNVPTLRPRSRMEVCESAV  
 35 HEPLHIEADPDLEPFSDFTFELDNTWGNADTWWKLGRNWGNSPHQGVGGCWESLRHILASGKKGV  
 REAPGLTSLFGLGQSVELLTLRSLPRGNLVPLFIGDKQGLSQKQTVHVRICPCASGLTCVELADAEV  
 GLHVGA LFVCAAFVALAVALLFLLRCYFVLEPKRHGCSVSNDGHTLVMYNAESKGTSAQTWSDVE  
 GQRPALLICTAAAGPTQGVKGREPKPPSRFWCISGFPSVSCKYCRSRGSASICSESVPSTLCSGEL  
 DSTETQIHRHGPDEQETASSPSWETMGSPAEWVLPGTCTTQTSSPGLEALPKSRQARLLQKGA VYP  
 40 QTQGCRA LPQVLTAELMGLEDRETEALGEAFMARLAADLKGDYLSL GREASTVESCVGRSQSPSH  
 WQAKKAWIPKLLQKRNFNNVAPIVNNVASIVNNIAPIVNNVAPIVNNVASIVNNVAPIVNNVAPIV  
 NIAPIVNNVASIVNNALQHFTLSARIHVQKVQSKERNRFSLSRGCIIPQGRATAGRGLPQDIYKEMMP  
 RRLTQTGKRKHGALARTPSFKKVYDHKEVSLICWVQTS PEDPPPHIPWIRTHQWFP SAWEF PFNGLR  
 TMSLPFLPEAQNP SYRSLPQRPSWASLQAFAYSVSSWSVPVPTPIYRNSTSPPGCPDGPRTGRLVYLP  
 45 RSRVGSGLAIMAEILLYLPLAAGALLTSSRVVNKELRMLSCP GTWLQVA

In a search of sequence databases, it was found, for example, that the nucleic acid  
 sequence has 225 of 381 bases (59%) identical to human cadherin-13 coding sequence (patn:  
 :T85405) (Table 6C).

```
>patn:T85405 Human cadherin-13 coding sequence - Homo sapiens, 2690 bp.
Score = 323 (48.5 bits), Expect = 6.4e-05, P = 6.4e-05
Identities = 225/381 (59%), Positives = 225/381 (59%), Strand = Plus / Plus
```

The full amino acid sequence of the protein was found to have 155 of 413 residues (37%), identical to, and 233 of 413 residues (56%) positive with, human neural-cadherin precursor (n-cadherin) having a total of 906 amino acid residues (SWISSPROT-ACC:P19022) (Table 6D).

40 >ptrn:SWISSPROT-ACC:P19022 NEURAL-CADHERIN PRECURSOR (N-CADHERIN) - Homo sapiens  
(Human), 906 aa.  
Score = 706 (248.5 bits), Expect = 1.3e-87, Sum P(3) = 1.3e-87  
Identities = 155/413 (37%), Positives = 233/413 (56%), Frame = +1

20



Sbjct: 480 --STATVSVTVIDVNENPYFAPNPKIIRQEEGLHAGTMLTTFTAQDPDRYMQQNIRYTKL 537

Query: 1387 HDPANWVSVDKNSGVVITVEPIDRESPHVNNISFYVIIHAVDDGFPPQTATGTLMLFLSD 1566  
 |||||+ +| +| +| +| |||||+| +| | +| || +| ||| ++| |

Sbjct: 538 SDPANWLKIDPVNGQITTIIVLDRESPNVKNNIYNATFLASDNGIPPMMSGTGTQLQIYLLD 597

Query: 1567 INDNVPTLRPRSRYMEVCESAVHEPLHIEADPDLEPFSDPFTFELDNTWGAEDTWKLG 1746  
 ||||| +| +| | ||+ ++| | | ++| +| | +| +| +| +| +|

Sbjct: 598 INDNAPQVLPQEA--ETCETPDPSINITALDYDIDPNAGPFAFDLPLSPVTIKRNWTIT 655

Query: 1747 R 1749  
 |

Sbjct: 656 R 656

A multiple sequence alignment for FCTR6 AL109928\_A is given in Table 6E, with the protein of the invention being shown on line 4, in a ClustalW analysis comparing the protein of the invention with related protein sequences.

**Table 6E.** BLASTX comparison of FCTR6 and human pre-N-cadherin (SEQ ID NO:44).

>ptnr:SWISSPROT-ACC:P19022 NEURAL-CADHERIN PRECURSOR (N-CADHERIN) - Homo sapiens (Human), 906 aa.  
 Score = 151 (53.2 bits), Expect = 1.3e-87, Sum P(3) = 1.3e-87  
 Identities = 31/82 (37%), Positives = 49/82 (59%), Frame = +1

Query: 157 LRSKRWRWVITTTLELEEDPGPFKLGELFNNMSYNMSLMYLSGPGVDEYPEIGLFSL 336  
 |+| || ||| +| | ||||+ + + ++ |+| | ++||| |+| |+| +

Sbjct: 154 LQRQKRDWVIPPINLPENSRGPFQELVIRSDRDKNLSLRYSVTGPGADQ-PPTGIFII 212

Query: 337 EDHENGRIYVHRPVDREMTSPF 402  
 +|++ | +|+||| |

Sbjct: 213 NPI-SGQLSVTKPLDREQIARF 233

The FCTR6 nucleotide sequence has two regions (nucleotides 1315-1757 and 1875-2305) identical to (100%) the 1808 bp human cadherin-like protein VR20 mRNA (VR20) (GenBank AF169690). Table 6F shows a partial BlastN alignment of FCTR6 with VR20.

**Table 6G.** BlastN alignment of FCTR6 nucleotide with VR20 mRNA (SEQ ID NO:45).

>Homo sapiens cadherin-like protein VR20 mRNA, partial cds (GenBank AF169690)

		1270	1280	1290	1300	1310	1320
40	FCTR6	.... .... .... .... .... .... .... .... .... .... .... ....					
	hVR20	AACGACCCACCAGCCTTTACCCCCAGAGCTTCATTGTCAATAAAGAGGAGGGCGCCAGG					CCAGG
		-----CCAGG					
		1330	1340	1350	1360	1370	1380
45	FCTR6	.... .... .... .... .... .... .... .... .... .... .... ....					
	hVR20	CCTGGGACCCCTGTTGGGAACCTTTAATGCCATGGATCCAGACAGCCAGATAAGATATGAA					CCTGGGACCCCTGTTGGGAACCTTTAATGCCATGGATCCAGACAGCCAGATAAGATATGAA
		CCTGGGACCCCTGTTGGGAACCTTTAATGCCATGGATCCAGACAGCCAGATAAGATATGAA					
		1390	1400	1410	1420	1430	1440
50	FCTR6	.... .... .... .... .... .... .... .... .... .... .... ....					
	hVR20	CTGGTTCATGACCCAGCAAATTGGGTTCAGCGTCGACAAAACTCCGGAGTGGTCATCACC					CTGGTTCATGACCCAGCAAATTGGGTTCAGCGTCGACAAAACTCCGGAGTGGTCATCACC
		CTGGTTCATGACCCAGCAAATTGGGTTCAGCGTCGACAAAACTCCGGAGTGGTCATCACC					
		1450	1460	1470	1480	1490	1500
55	FCTR6	.... .... .... .... .... .... .... .... .... .... .... ....					
	hVR20	GTGGAGCCAATTGACCGAGAATCCCCTCATGTAAATAACAGTTTTTATGTAATCATCATT					GTGGAGCCAATTGACCGAGAATCCCCTCATGTAAATAACAGTTTTTATGTAATCATCATT
		GTGGAGCCAATTGACCGAGAATCCCCTCATGTAAATAACAGTTTTTATGTAATCATCATT					
		1510	1520	1530	1540	1550	1560

22

Table 6G shows a ClustalW alignment of FCTR6 with related proteins found in public databases. FCTR6 polypeptide is on line 5, human CAD2 (SEQ ID NO:46) is on line 1, bovine CAD2 (SEQ ID NO:47) is on line 2, mouse CAD2 (SEQ ID NO:48) is on line 3, and chicken CAD2 (SEQ ID NO:49) is on line 4. Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

**Table 6G.** ClustalW alignment including FCTR6 (AL109928\_A) protein.

	CAD2_HUMAN	MCRIAGALRTLLP-----LLLAALQASVPASGEIALCKTGFPEDVYSAVLSKDVHEGQPL
	CAD2_BOVIN	-----SLCKTGFPEDVYSAVLSRDVLEGQPL
	CAD2_MOUSE	MCRIAGGRGTLLP-----LLAALQASVPASGEIALCKTGFPEDVYSAVLPKDVHEGQPL
15	CAD2_CHICK	MCRIAGTPPRLLPPLALMLLAALQAPKATCEDMLCKMGFPEDVHSASVSRSSVHGQPL
	AL109928_genscan2	_MRSG-----RHP-----SLLLLLVLLWLLQVSIISVQQETD
	CAD2_HUMAN	LNVKFSNCNCKRRKVQYESSEPADFKVDEDEGVYAVRSFPLSSE-HAKFLIVAQDKETQEK
	CAD2_BOVIN	LNVKFSNCNCKRRKVQYESSEPADFKVDEDEGVYAVRSFPLSSE-HSKFLIYAQDKETQEK
20	CAD2_MOUSE	LNVKFSNCNCKRRKVQYESSEPADFKVDEDEGVYAVRSFPLTAE-QAKFLIYAQDKETQEK
	CAD2_CHICK	LNVRFSQCDENRKITYEGSSEPEDERVGEDGVYAESEFOLSAE-PTFVVSARDKETQEE
	AL109928_genscan_2	DLTKQTKCH--YKFEKIYQP--LRSKR--RWVTITLELEEDDPGPPFKLIGELFNMS
	CAD2_HUMAN	WQVAVKLSLKEPTLTESVKESAEVEEIVFPQFQSKHSGHLQROKRDWVIPPINLPENSRG
	CAD2_BOVIN	WQVAVKLSLKEPALPEESVKESREIEEIVFPQVTKHNGYLQROKRDWVIPPINLPENSRG
25	CAD2_MOUSE	WQVAVNLSREPTLTETPEMKEPHEIEEIVFPQQLAKHSCALQROKRDWVIPPINLPENSRG
	CAD2_CHICK	WQMKVKLTPEPAFTGASEKDKKIEDIIFWQYKDSHLLKROKRDWVIPPINLPENSRG
	AL109928_genscan_2	YNMSLMYLTIS---GPGVDEYPEIG--LESLEDHENG-----RIYVHRPVDR-EMTPS
	CAD2_HUMAN	PFPQELVIRISDRDKNLSLRYSVTGPGADQPPTGIFINPISGQLSVTKPLDRELIARFH
	CAD2_BOVIN	PFPQELVIRISDRDKNLSLRYSVTGPGADQPPTGIFINPISGQLSVTKPLDRELIARFH
	CAD2_MOUSE	PFPQELVIRISDRDKNLSLRYSVTGPGADQPPTGIFINPISGQLSVTKPLDRELIARFH
	CAD2_CHICK	PFPQELVIRISDRDKSLSLRYSVTGPGADQPPTGIFINPISGQLSVTKPLDRELIASEH
30	AL109928_genscan_2	-FTSWTARVPSR-----ASAGMSR-----GHL-----REGLVYVYFD
	CAD2_HUMAN	LRAHAVDINGNQVENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA
	CAD2_BOVIN	LRAHAVDINGNQVENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA
	CAD2_MOUSE	LRAHAVDINGNQVENPIDIVINVIDMNDNRPEFLHQVWNGSVPEGSKPGTYVMTVTAIDA
	CAD2_CHICK	LRAHAVDINGNQVENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA
40	AL109928_genscan_2	VVERST---CKLVDTSEIFNIRISDVNDHAEQFPEKEENITVQENQASAGQPIFQMLAVDL
	CAD2_HUMAN	DDPNALNGMLRYRILVSOAPSTSPSPNMFTINNETGDIITVAAGLDREKVQOQYTLIIQATDM
	CAD2_BOVIN	DDPNALNGMLRYRILVSOAPSTSPSPNMFTINNETGDIITVAAGLDREKVQOQYTLIIQATDM
	CAD2_MOUSE	DDPNALNGMLRYRILVSOAPSTSPSPNMFTINNETGDIITVAAGLDREKVQOQYTLIIQATDM
45	CAD2_CHICK	DDPNAQNGMLRYRILVSOAPSSPSPNMFTINNETGDIITVAAGLDREKVQOQYTLIIQATDM
	AL109928_genscan_2	DEBNTPNISQVLYFETISQTEPLLK-ESGFRVDRLSGEIR-MSGCLDYETAPQETLEIRARDC
	CAD2_HUMAN	EGNPTYGLSNTATAVITVTDWNDNPPEETAMTFYGEVPEENRVDIVANLTVTDKDPHTP
	CAD2_BOVIN	EGNPTYGLSNTATAVITVTDWNDNPPEETAMTFYGEVPEENRVDIVANLTVTDKDPHTP
	CAD2_MOUSE	EGNPTYGLSNTATAVITVTDWNDNPPEETAMTFYGEVPEENRVDIVANLTVTDKDPHTP
50	CAD2_CHICK	EGNPTYGLSNTATAVITVTDWNDNPPEETAMTFYGEVPEENRVDIVANLTVTDKDPHTP
	AL109928_genscan_2	-GEPSS--LSSTTTTHVDVQEGNNHRPAFTQENYKVOQIPEGRASQCVLRLLVQDRDSPTFS



	CAD2_HUMAN	-----
	CAD2_BOVIN	-----
	CAD2_MOUSE	-----
	CAD2_CHICK	-----
5	AL109928_genscan_2	VNNVAPIVNNVASIVNNVAPIVNNVAPIVNNIAPIVNNVASIVNNALQHFTLSARIHVQK
	CAD2_HUMAN	-----
	CAD2_BOVIN	-----
	CAD2_MOUSE	-----
10	CAD2_CHICK	-----
	AL109928_genscan_2	VQSKERNRFSLSRGCIIPQGRATAGRGLPQDIYKEMMPRRLTQTGKRKHGALARTPSFKK
	CAD2_HUMAN	-----
	CAD2_BOVIN	-----
15	CAD2_MOUSE	-----
	CAD2_CHICK	-----
	AL109928_genscan_2	VVYDHKEVSLICWVQTSPEDPPPHIPWIRTHQWFPSAWEFPFNGLRRTMSLPGLPEAQNPS
	CAD2_HUMAN	-----
20	CAD2_BOVIN	-----
	CAD2_MOUSE	-----
	CAD2_CHICK	-----
	AL109928_genscan_2	YRSLPQRPSWASLQAFAYSVPSSWSVPVPTPIYRNSTSPPGCPDGPRTGRLVYLPRSRVGS
25	CAD2_HUMAN	-----
	CAD2_BOVIN	-----
	CAD2_MOUSE	-----
	CAD2_CHICK	-----
30	AL109928_genscan_2	GPLAIMAEILLYPLAAGALLTSSRVVNKELRMLSCP GTWLQVA

From these analyses, it is seen that the FCTR6 AL109928\_A nucleic acid and protein a weak resemblance to neural cadherin, and a strong resemblance across a portion of FCTR6 with human cadherin-like VR20. Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. N-cadherin may be involved in neuronal recognition mechanism. They are type I membrane proteins.

Finally, FCTR6 was found to have high homology to the domains shown in Table 6H.

**Table 6H: CD domain analysis of FCTR4**

Sequences producing significant alignments:	Score (bits)	E value
cadherin, Cadherin domain	73.9	5e-14
cadherin, Cadherin domain	57.0	6e-09
cadherin, Cadherin domain	44.3	4e-05
cadherin, Cadherin domain	40.4	6e-04
Cadherin repeats.; Cadherins are glycoproteins involved in Ca2...	56.6	8e-09
Cadherin repeats.; Cadherins are glycoproteins involved in Ca2...	49.3	1e-06

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

**FCTR7 (AL109953\_A)**

The novel FCTR7 nucleic acid encoding a novel secreted FCTR7 protein is shown in Table 7A1. This sequence contains an initiation codon at the 5' end, and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. An alternative novel FCTR7A nucleic acid encoding a novel secreted protein is shown in Table 7A2. This sequence contains an initiation codon at the 5' end, a frameshift at position 61, and a TAA stop codon indicating that this sequence is a coding sequence. The start and stop codons for both sequences are shown in bold letters. These sequences originate in chromosome 20 clone RP4-746H2.

**Table 7A1. FCTR7 (AL109953\_A) nucleotide sequence (SEQ ID NO:13).**

atgggatgcagactgctgacctgctgtgtttcctacaacctgcttccagctcctcgtgggtctttgg  
ctcccaatccagagctttcggaacaccagagccccctgtgcctctccctgcagctgggtgggagttcc  
agggcattaacacagacagctctttgcccatcagccagtgactgtatggagcttggatgtgaatacaca  
gctcctgcatccctccgaggcatctccacaccgtctcccagagaatgtctcgtaaaagctgctcctct  
tggggagggtctgggctttggagagagcacctggaattccccactagaaaagcccaaaaactga

**Table 7A2. Alternative FCTR7A (AL109953\_A) nucleotide sequence (SEQ ID NO:29).**

atgggatgcagactgctgacctgctgtgtttcctacaacctgcttccagctcctcgtgggtctttggc  
tcccaatccagagctttcggaacaccagagccccctgtgcctctccctgcagctgggtgggagttcca  
gggcattaacacagacagctctttgcccatcagccagtgactgtatggagcttggatgtgaatacaca  
ctcctgcatccctccgaggcatctccacaccgtctcccagagaatgtctcgtaaaagctgctcctctt  
ggggagggtctgggctttggagagagcacctggaattccccactagaaaagcccaaaaactga

The encoded FCTR7 protein is presented using the one-letter code in Table 7B1. The FCTR7 protein has a low probability of being secreted extracellularly, although a signal peptide most likely is cleaved between residues 17 and 18, *i.e.* at the dash in the sequence ASS-SSW. The encoded FCTR7A protein is presented using the one-letter code in Table 7B2.

**Table 7B. FCTR7 protein sequence (SEQ ID NO:14) encoded by SEQ ID NO:13.**

MGCRLLTLLCFLQPASSSSWLFQSRAFANTRAPVPLPAAGWEFQGINTDSLCPASDCMELGCEYT  
APASLRGISTPSPRECLVKAAPLGEALGFGESEWNSPLEKPKN

**Table 7B. FCTR7A protein sequence (SEQ ID NO:30) encoded by SEQ ID NO:29.**

MGCRLLTLLCFLQPASSSSWLSLAPNPESRTPEPLCLSLQLAGSSRALTQTVFAHQPVTVWSLDVNTQ  
LLHPSEASPHRLPENVS

In a search of sequence databases, no similarities were found to known nucleic acid or protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR8 (AL110115\_A)

5 The novel nucleic acid encoding a novel secreted protein is shown in Table 8A. This sequence contains an initiation codon at the 5' end, and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in chromosome 20 clone RP3-324O17.

**Table 8A.** FCTR8 (AL110115\_A) nucleotide sequence (SEQ ID NO:15).

10 **atgaagctccttcttctgcttttgactgttactctgctcctggcccaggtcaccccaggtctgccagc**  
**catgaaacttctttacctgtttcttgccatccttctggccatagaagaaccagtgatatcagtagagt**  
**gttgatggatggacactgccggtgtgtgtgcaaagatggtgaagacagcatcatacgctgccgaaat**  
**cgtaaacggtgctgtgttcctagtcgttatttaacaatccaaccagtaacaattcatggaatccttgg**  
**ctggaccactcctcagatgtccacaacagctccaaaatgaagacaaatataactaatagatag**

15 The encoded protein is presented using the one-letter code in Table 8B. The protein has a moderate probability of sorting to the plasma membrane. A signal peptide most likely is cleaved between residues 43 and 44, *i.e.* at the dash in the sequence VIS-VEC.

**Table 8B.** Encoded FCTR8 protein sequence (SEQ ID NO:16).

20 **MKLLLLLLTVTLLLAQVTPGLPAMKLLYLFLAILLAIEEPVISVECWMDGHCRLCLKDGEDSIIRCRN**  
**RKRCCVPSRYLTIQPVTIHGILGWTTTPQMSTTAPKMKTNITNR**

In a search of sequence databases, the BLASTN comparison revealed 91 of 129 bases (70%), out of a total of 413 bases, are identical to an unidentified human secreted protein. No similarities of significance were identified at the amino acid level.

**Table 8C.** BLASTN of FCTR8 with (SEQ ID NO:50).

Query: 28 ATGAAGCTCCTTCTTCTGCTTTTGACTGTTACT-CTGCTCCTGGCCCAGGTCACCCCAGG 86  
 |||||  
 Sbjct: 43 ATGAAGCTCCTTTTGCTGACTTTGACTGTG-CTGCTGCTCTTATCCCAGCTGACTCCAGG 101  
 Query: 87 TCTGCCAGCCATGAACTTCTTTACCTGTTTCTTGCCA-T-CC-TT-CTG--GC-CATAG 139  
 |||||  
 Sbjct: 102 TG-GC-ACCCAA-AGA-TGCTGGAA-TCTTTATGGCAAATGCCGTTACAGATGCTCCAAG 156  
 Query: 140 AAG-AACCAGTGATAT 154  
 |||||  
 Sbjct: 157 AAGGAAAGAGTC-TAT 171

40 The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

**FCTR9 (AL117336\_A)**

The novel nucleic acid encoding a novel secreted protein is shown in Table 9A. This sequence contains an initiation codon at the 5' end, and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are indicated in bold type. This sequence originates in chromosome 10 clone RP11-324I22.

**Table 9A.** FCTR9 (AL117336\_A) nucleotide sequence (SEQ ID NO:17).

**at**ggcaaaggagggggccccaggagcccttgagaccgctgggcttgctgcctccccgcattctggccca  
gtgctgcttggtcactctggctgtgcctccagcaggcccagctctcaacgctggctgcacgggtcaaga  
cctag

The encoded protein is presented using the one-letter code in Table 9B. The protein has a moderate probability of sorting to the plasma membrane. A signal peptide most likely is cleaved between residues 43 and 44, *i.e.*, the dash in the amino acid sequence GCT-VKT.

**Table 9B.** Encoded FCTR9 protein sequence (SEQ ID NO:18).

MAKEGPQEPLRPLGLLPRI LAQCCLVTLAVPPAGPALNAGCTVKT

In a search of sequence databases no similarities of significance were identified at either the nucleic acid or the amino acid level.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

**FCTR10 (AL118509\_A)**

The novel nucleic acid encoding a novel secreted protein is shown in Table 10A. This sequence contains an initiation codon at the 5' end, and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type. This sequence originates in chromosome 20 clone RP4-770C23.

**Table 10A.** FCTR10 (AL118509\_A) nucleotide sequence (SEQ ID NO:19)

**at**gcactcactgcggttcctactgcttttggtggttgctgtttcctctgtcactgctatccttctcttc  
ccctacagtaggggtttctggactgcggcacagttgtcacttcagaccaggtaagggctctattaatta  
tggtctatgaatcacaatcagatttaaaaaacaaacaaaaataaaacaaaacaaaaacaaaaagagaa  
gggaaggagcggctctgtgaacgttaacaaatggaaatccactggggatcagcctctgtcagaactaag  
ctccaggaaggaggaggttcagccagttgaggagccagtatcattatcagaaggggaatttaggaaaaa  
gcaagaaggtgatgaagaatgagagggaggaagaaaagaaggaaaaggaacaaacttccagcttctca  
caattcccttctgaaagacgtacactgcccattggcaaggcacgctggatatgggttaagtaaccccaa  
tctgaaaatccaaaatccaaaatgctacaacatcccaaagtgttttgagtgccaatgtgatgatcaatg  
gaaatgttcactag



The encoded protein is presented using the one-letter code in Table 10B. The protein has a high probability of being secreted extracellularly. A signal peptide most likely is cleaved between residues 27 and 28, *i.e.* at the dash in the sequence TVG-FLD.

**Table 10B.** Encoded FCTR10 protein sequence (SEQ ID NO:20).

5 MHSLRFLLLLWLLFPLSLLSFSSPTVGFLDCGTVVTSQVRRALLIMFYESQSDLKTNKNKTKQKQKRE  
GKERSVNVNWKWKSTGDQPLSELSSRKEEVQPVVEPVSLSEGNLKGSKKVMKNEREEEEKKEKEQTSSFS  
QFPSERRTLPMARHAGYGLSNPNLKIQNPKCYNIPNVLSANVMINGNVH

In a search of sequence databases, the BLASTN comparison (see Table 10C)  
10 revealed 90 of 117 bases (76%), in a large genomic fragment originating on chromosome  
6q23.1-24.3, are identical to a human DNA sequence containing the MEKK5 (ASK1,  
MAPKKK5) gene for MAP/ERK kinase kinase 5 (Mitogen Activated Protein kinase kinase  
kinase 5), as well as ESTs, GSSs and a putative CpG island. No similarities of significance  
were identified at the amino acid level.

**Table 10C.** BLASTN of FCTR10 with MEKK5.

15 >gb:GENBANK-ID:HS325F22|acc:AL024508 Human DNA sequence from clone 325F22 on  
chromosome 6q23.1-24.3. Contains the MEKK5 (ASK1, MAPKKK5) gene for MAP/ERK kinase  
kinase 5 (Mitogen Activated Protein kinase kinase kinase 5), ESTs, GSSs and a  
putative CpG island, complete sequence - Homo sapiens, 154788 bp.  
20 Score = 330 (49.5 bits), Expect = 1.9e-10, Sum P(2) = 1.9e-10  
Identities = 90/117 (76%), Positives = 90/117 (76%), Strand = Plus / Plus

Query: 444 AAGGCACGCTGGATATGG--GTTAAGTAACCCCAATCTGAAAATCCAAAATCCAAAATGC 501  
||||| ||| || ||| ||||| ||| ||||| ||||| |||||  
25 Sbjct: 145356 AAGGCACACTGTAATAACAAGTTGAGTAACCCCTAATAAAAAAATCTGAAATCTAAATGC 145415

Query: 502 TACAACATCCCAAATGTTTTGAGTGCCCAATGTGATGATCAATGGAATGTTCACTAG 558  
| ||| |||| ||| ||||| ||||| ||||| ||||| ||||| ||| |  
30 Sbjct: 145416 TCCAAAATCCCAAATCTTTTGGAGTGCCCAATGATGCTCAAAGGAAATGCTCATTGG 145472 (SEQ  
ID NO:51)

Score = 163 (24.5 bits), Expect = 1.9e-10, Sum P(2) = 1.9e-10  
Identities = 69/96 (71%), Positives = 69/96 (71%), Strand = Plus / Plus  
35 Query: 145 GAATCACAATCAGATTAAAAACAAACAAAAATAAAA-CAAAA-CAAAAACAAAAAGAG 202  
||| ||| ||| ||| ||||| ||||| ||||| ||||| ||||| ||||| |||  
Sbjct: 29978 GAGTGAGACTCCG-TCTCAAAA-AAACAAAAACAAAAACAAAAACAAAAACAAAG 30035

40 Query: 203 AAGGGAAGGAGCGGTCTGTGAACGTTAACAAATGGAAA 240  
|| ||| ||| ||| ||||| ||||| ||||| ||||| |||  
Sbjct: 30036 AA---ATGCATCCATAT-T-AAC-TTC-CAAATGCAAA 30066 (SEQ ID NO:52)

45 Score = 121 (18.2 bits), Expect = 1.4e-08, Sum P(2) = 1.4e-08  
Identities = 57/86 (66%), Positives = 57/86 (66%), Strand = Plus / Plus

Query: 94 GGCACAGTTGTCTACCTCAGACCAGGT-A-AGGGCTCTATT-AATTATGTTCTATGAATCA 150  
||||| ||| |||| ||| ||| ||| ||| ||| ||| ||| |||  
50 Sbjct: 13513 GGCACATTTT-ACTTT-GAGGTGATTACATTGCTTTACTCAAAGAACTGGTGGAAATGG 13570

Query: 151 CAATCAGATTTAAAAACAAACAAAAATAA 179  
| | ||| ||||| ||||| ||||| ||||| ||||| |||  
55 Sbjct: 13571 CTAA-AGTTTAAAAACAAACAAACTAA 13598 (SEQ ID NO:53)

Score = 117 (17.6 bits), Expect = 2.0e-08, Sum P(2) = 2.0e-08  
Identities = 27/30 (90%), Positives = 27/30 (90%), Strand = Plus / Plus

5 Query: 171 CAAAAATAAAACAAAACAAAACAAAAAAG 200  
|||||  
Sbjct: 101741 CAAAAATAAAACAAAACAAAAG-AATAAAG 101769 (SEQ ID NO:54)

10 The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR11 (AL118522\_A\_EXT)

15 The novel nucleic acid encoding a novel K<sup>+</sup> channel-like protein is shown in Table 11A. This sequence contains an initiation codon at the 5' end, and a TAA stop codon was identified near the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type and a putative 3'UTR is underlined. This sequence originates in chromosome 20 and was assembled as a consensus extension using the 8 sequences FCTR11 AL118522\_genscan\_2+, est:gb\_AA283204+, est:gb\_AI091631-, est:gb\_AI097455+, est:gb\_AI690321+, est:gb\_AI739096+, est:gb\_AI968607+, and  
20 est:gb\_AW073155+.

**Table 11A.** FCTR11 (AL118522\_A\_EXT) nucleotide sequence (SEQ ID NO:21).

25 **ATGCGGAGGCGGAGCGTGCGCGCGGCGGGCTGGTCTGTGCACCCCTGTGTTACCTGCTGGTGGGCGC**  
TGCTGTCTTCGACGCGCTCGAGTCCGAGGCGGAAAGCGGCCGCGAGCGACTGCTGGTCCAGAAGCGGG  
GCGCTCTCCGGAGGAAGTTCGGCTTCTCGGCCGAGGACTACCGCGAGCTGGAGCGCCTGGCGCTCCAG  
30 GCTGAGCCCCACCGCGCCGCGCCAGTGGAAAGTTCCCCGGCTCCTTCTACTTCGCCATCACCGTCAT  
CACTACCATCGAGTACGCCACGCCGCGCCGGGTACGGACTCCGGCAAGGTCTTCTGCATGTTCTACG  
CGCTCCTGGGCATCCCGCTGACGCTGGTCACTTTCCAGAGCCTGGGCGAACGGCTGAACGCGGTGGTG  
CGGCGCCTCCTGTTGGCGGCCAAGTGCTGCCTGGGCTGCGGTGGACGTGCGTGTCCACGGAGAACCT  
GGTGGTGGCCGGGCTGCTGGCGTGTGCCGCCACCCCTGGCCCTCGGGGCCGTGCGCTTCTCGCACTTCG  
35 AGGGCTGGACCTTCTTCCACGCCCTACTACTGCTTCATCACCCCTCACCACCATCGGCTTCGGCGAC  
TTCGTGGCACTGCAGAGCGGCGAGGCGCTGCAGAGGAAGCTCCCCTACGTGGCCTTCAGCTTCCTCTA  
CATCCTCCTGGGGCTCACGGTCATTGGCGCCTTCTCAACCTGGTGGTCTCTGCGCTTCTCGTTGCCA  
GCGCCGACTGGCCCCGAGCGCGCTGCCCGCACCCCCAGCCCGCGCCCCCGGGGGCGCCGAGAGCCGT  
GGCCTCTGGCTGCCCCGCGCCGCGCCGCTCCGTGGGCTCCGCCTCTGTCTTCTGCCACGTGCACAA  
40 GCTGGAGAGGTGCGCCCGGACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCGTGCGTGGCG  
GGCAGGCTCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCCAGGCCAGGGTCGAATC  
TGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCCCTCCCCAGGGATTGGAACGGATGACGGGCC  
TTTAGGCGGTTTTTTGCCACGAGCAGTTTTTCACTACTGTCTGTGGCTAAGTCCCCTCCCTCCTTTCC  
AAAAATATATTACAGTCACCCCAT**AAGCCCAAAAAAAAAAAAAAAAAAAAAAAAAA**

The encoded protein is presented using the one-letter code in Table 11B. The protein has a high probability of being sorted to the plasma membrane. A signal peptide most likely is cleaved between residues 23 and 24, *i.e.* at the dash in the sequence VGA-AVF.

MRRPSVRAAGLVLCTLCYLLVGAAVFDALSEAESESGRQRLLVQKRGALRRKFGFSAEDYRELERLALQ  
AEPHRAGRQWKFPGSFYFAITVITTIIEYGHAAPGTDSGKVF CMFYALLGIPLTLVTFQSLGERLNAV  
RRLLLAAKCCLGLRWTCVSTENLVVAGLLACAATLALGAVAFSHFEGWTFHAYYYCFITLTTIGFGD  
FVALQSGEALQRKLPYVAFSFLYILLGLTVIGAFNLVLRFLVASADWP ERAARTPSRPPGPAPESR  
GLWLPRRPARSVGSASVFCHVHKLERCARDNLGFSPPSSPGVVRGGQAPRLGARWKSIXQPHPGQGRI  
WNGRVWLQLSGHPPOGLETDDGPLGGFLPRAVFHYCLWLSPLPPFOKYITVTP

10  
15

```
>gb:GENBANK-ID:AF006823|acc:AF006823 Homo sapiens TWIK-related acid-sensitive K+
channel (TASK) mRNA,
    complete cds - Homo sapiens, 2590 bp (RNA).
Score = 2097 (314.6 bits), Expect = 4.2e-89, P = 4.2e-89
Identities = 641/854 (75%), Positives = 641/854 (75%), Strand = Plus / Plus
```

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25  
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FCTR11 CCGCGAGCTGGAGCGCTGGCGCTCCAGGCTGAGCCCCACGCGCCGGCCGCCAGTGGAA  
 Line 2 CCGCGAGCTGGAGCGCTGGCGCTCCAGGCTGAGCCCCACGCGCCGGCCGCCAGTGGAA  
 Line 3 CCGCGAGCTGGAGCGCTGGCGCTCCAGGCTGAGCCCCACGCGCCGGCCGCCAGTGGAA

5 310 320 330 340 350 360

FCTR11 GTTCCCCGGCTCCTTCTACTTCGCCATCACCGTCATCACTACCATCGGAGTACGGCCACGC  
 Line 2 GTTCCCCGGCTCCTTCTACTTCGCCATCACCGTCATCACTACCATCGGGTACGGCCACGC  
 Line 3 GTTCCCCGGCTCCTTCTACTTCGCCATCACCGTCATCACTACCATCGGGTACGGCCACGC

10

370 380 390 400 410 420

FCTR11 CGCGCCGGGTACGGACTCCGGCAAGGTCTTCTGCATGTTCTACGCGCTCCTGGGCATCCC  
 Line 2 CGCGCCGGGTACGGACTCCGGCAAGGTCTTCTGCATGTTCTACGCGCTCCTGGGCATCCC  
 Line 3 CGCGCCGGGTACGGACTCCGGCAAGGTCTTCTGCATGTTCTACGCGCTCCTGGGCATCCC

15

430 440 450 460 470 480

FCTR11 GCTGACGCTGGTCACTTTCAGAGCCTGGGCGAACGGCTGAACGCGGTGGTGGCGCGCCT  
 Line 2 GCTGACGCTGGTCACTTTCAGAGCCTGGGCGAACGGCTGAACGCGGTGGTGGCGCGCCT  
 Line 3 GCTGACGCTGGTCACTTTCAGAGCCTGGGCGAACGGCTGAACGCGGTGGTGGCGCGCCT

20

490 500 510 520 530 540

FCTR11 CCTGTTGGCGGCCAAGTGCTGCCTGGGCGTGCCTGGACGTGCGTGTCCACGGAGAACCCT  
 Line 2 CCTGTTGGCGGCCAAGTGCTGCCTGGGCGTGCCTGGACGTGCGTGTCCACGGAGAACCCT  
 Line 3 CCTGTTGGCGGCCAAGTGCTGCCTGGGCGTGCCTGGACGTGCGTGTCCACGGAGAACCCT

25

550 560 570 580 590 600

FCTR11 GGTGGTGGCCGGGCTGCTGGCGTGTGCCGCCACCCTGGCCCTCGGGGCCGTGCGCTTCTC  
 Line 2 GGTGGTGGCCGGGCTGCTGGCGTGTGCCGCCACCCTGGCCCTCGGGGCCGTGCGCTTCTC  
 Line 3 GGTGGTGGCCGGGCTGCTGGCGTGTGCCGCCACCCTGGCCCTCGGGGCCGTGCGCTTCTC

30

610 620 630 640 650 660

FCTR11 GCACTTCGAGGGCTGGACCTTCTCCACGCCTACTACTACTGCTTCATCACCCCTACCAC  
 Line 2 GCACTTCGAGGGCTGGACCTTCTCCACGCCTACTACTACTGCTTCATCACCCCTACCAC  
 Line 3 GCACTTCGAGGGCTGGACCTTCTCCACGCCTACTACTACTGCTTCATCACCCCTACCAC

35

670 680 690 700 710 720

FCTR11 CATCGGCTTCGGCGACTTCGTGGCACTGCAGAGCGCGAGGCGCTGCAGAGGAAGCTCCC  
 Line 2 CATCGGCTTCGGCGACTTCGTGGCACTGCAGAGCGCGAGGCGCTGCAGAGGAAGCTCCC  
 Line 3 CATCGGCTTCGGCGACTTCGTGGCACTGCAGAGCGCGAGGCGCTGCAGAGGAAGCTCCC

45

730 740 750 760 770 780

FCTR11 CTACGTGGCCTTCAGCTTCCTCTACATCCTCCTGGGGCTCACGGTCATTGGCGCCTTCCT  
 Line 2 CTACGTGGCCTTCAGCTTCCTCTACATCCTCCTGGGGCTCACGGTCATTGGCGCCTTCCT  
 Line 3 CTACGTGGCCTTCAGCTTCCTCTACATCCTCCTGGGGCTCACGGTCATTGGCGCCTTCCT

50

790 800 810 820 830 840

FCTR11 CAACCTGGTGGTCTGCGCTTCCTCGTTGCCAGCGCGACTGGCCCGAGCGCGCTGCCCG  
 Line 2 CAACCTGGTGGTCTGCGCTTCCTCGTTGCCAGCGCGACTGGCCCGAGCGCGCTGCCCG  
 Line 3 CAACCTGGTGGTCTGCGCTTCCTCGTTGCCAGCGCGACTGGCCCGAGCGCGCTGCCCG

55

850 860 870 880 890 900

FCTR11 CACCCCGAGCCCGCGCCCCCGGGGGCGCCGAGAGCCGTGGCTCTGGCTGCCCGCCCG  
 Line 2 CACCCCGAGCCCGCGCCCCCGGGGGCGCCGAGAGCCGTGGCTCTGGCTGCCCGCCCG  
 Line 3 CACCCCGAGCCCGCGCCCCCGGGGGCGCCGAGAGCCGTGGCTCTGGCTGCCCGCCCG

60

910 920 930 940 950 960

FCTR11 CCGGGCCCGCTCCGTGGGCTCCGCCTCTGTCTTCTGCCACGTGCACAAGCTGGAGAGGTG  
 Line 2 CCGGGCCCGCTCCGTGGGCTCCGCCTCTGTCTTCTGCCACGTGCACAAGCTGGAGAGGTG

65

0746491-074301

Line 3 CCGGCCCGCTCCGCTGGGCTCCGCTCTGTCTTCTGCCACGTGCACAAGCTGGAGAGGTG

970 980 990 1000 1010 1020

5 FCTR11 CGCCCGCGACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCGTGCGTGGCGGGCA  
Line 2 CGCCCGCGACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCGTGCGTGGCGGGCA  
Line 3 CGCCCGCGACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCGTGCGTGGCGGGCA

1030 1040 1050 1060 1070 1080

10 FCTR11 GGCTCCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCCAGGCCAGGGTCG  
Line 2 GGCTCCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCCAGGCCAGGGTCG  
Line 3 GGCTCCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCCAGGCCAGGGTCG

1090 1100 1110 1120 1130 1140

15 FCTR11 AATCTGGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCCCTCCCAGGGATTGGAAC  
Line 2 AATCTGGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCCCTCCCAGGGATTGGAAC  
Line 3 AATCTGGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCCCTCCCAGGGATTGGAAC

1150 1160 1170 1180 1190 1200

20 FCTR11 GGATGACGGGCTTAGGCGGTCTTGTGCAACGAGCA-GTTTTCATTACTGTCTGTGGC  
Line 2 GGATGACGGGCTTAGGCGGTCTTGTGCAACGAGCAAGTTTTCATTACTGTCTGTGGC  
Line 3 GGATGACGGGCTTAGGCGGTCTTGTGCAACGAGCAAGTTTTCATTACTGTCTGTGGC

1210 1220 1230 1240 1250 1260

30 FCTR11 TAAGTCCCTCCCTCTTTCCAAAAATATATTACAGTCACCCCATAGGCCAAAAAAAAA  
Line 2 TAAGTCCCTCCCTCTTTCCAAAAATATATTACAGTCACCCCATAAAAAAAAAAAAA  
Line 3 TAAGTCCCTCCCTCTTTCCAAAAATATATTACAGTCACCCCATAAAAAAAAAAAAA

1270 1280

35 FCTR11 AAAAAAAAAAAAAAAAAAAAAA-----  
Line 2 AAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:56)  
Line 3 AAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:57)

A BlastP search against the FCTR11 protein also identified FCTR11 as having high  
homology to the potassium channel proteins TASK and KT3.3, as shown in Table 11E. Line  
1 shows the FCTR11 polypeptide (SEQ ID NO:22), line 2 is the human TASK protein  
(gi|10944275|emb|CAC14068.1| (AL118522) dJ781B1.1 (A novel protein similar to the acid  
sensitive potassium channel protein TASK (KCNK3)) [Homo sapiens])(SEQ ID NO:58), line  
3 is the human KT3.3 protein (gi|11228686|gb|AAG33127.1|AF257081\_1 (AF257081) two  
pore potassium channel KT3.3 [Homo sapiens]) (SEQ ID NO:59), and line 4 is the guinea pig  
TASK3 protein (gi|7546839|gb|AAF63706.1|AF212827\_1 (AF212827) potassium channel  
TASK3 [Cavia porcellus])(SEQ ID NO:60).

Table 11E: BlastP search of FCTR11 protein

10 20 30 40 50 60

FCTR11 MRRPSVRAAGLVLCITLCYLLVGA AVFDALESEAESGRQRLLVQKRGALRRKFGFSAEDYR  
Line 2 MRRPSVRAAGLVLCITLCYLLVGA AVFDALESEAESGRQRLLVQKRGALRRKFGFSAEDYR  
Line 3 MRRPSVRAAGLVLCITLCYLLVGA AVFDALESEAESGRQRLLVQKRGALRRKFGFSAEDYR  
Line 4 MKKQNVRTLSTLACTFTYLLVGA AVFDALESDHEMREBEKLEIRIRKYNISTEDYR

70 80 90 100 110 120

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**Table 11F. BLASTX of FCTR11 nucleotide with CTBAK.**

Score = 832 (292.9 bits), Expect = 1.0e-85, Sum P(2) = 1.0e-85

Score = 832 (292.9 bits), Expect = 1.0e-85, Sum P(2) = 1.0e-85

45

50

Query: 178 RELERLALQAEPHRAGRQWKFPGSFYFAITVITTIEYGHAAPGTDSGKVFCEMFYALLGIP 357

Sbjct: 60 EELERVVLRLKPHKAGVQWRFAGSFYFAITVITTIGYGHAAPSTDGGKVFCMFYALLGIP 119

55

Query: 358 LTLVTFQSLGERLNAVVRLLLAACCLGLRWTCVSTENLVVAGLLACAATLALGAVAFS 537

Sbjct: 120 LTLVMFQSLGERINTFVRYLLHRAKRGGLMRHAEVSMANMVLIGFVSCISTLCIGAAAFS 179

60

Query: 538 HFEGWTFHAYYYCFITLTTIGFGDFVALQSGEALQRKLPYVAFSFLYLGLTVIGAFL 717  
++| | | | | | | | | | + | | | + | | | + | | |

Sbjct: 180 YYERWTFQAYYYCFITLTTIGFGDYVALQKDQALQTQPQYVAFSFVYILTGLTVIGAF 239

Query: 718 NLVVLRLFLVASADWPERAA 774

65

Sbict: 240 NLVVLRFMTMNAEDEKRDA 258 (SEO ID NO:61)

Score = 52 (18.3 bits), Expect = 1.0e-85, Sum P(2) = 1.0e-85  
Identities = 17/35 (48%), Positives = 20/35 (57%), Frame = +2

Query: 941 SCVAGRLPGLPGGSPSDNPTQAR---VESGMGGSGF 1042  
| | + + | | | | | | | | + + | | | |  
Sbjct: 277 SCLSG---SLGDGVRPRDPVTCAAAAGVGVGVGSGSF 311 (SEQ ID NO:62)

Score = 40 (14.1 bits), Expect = 1.9e-84, Sum P(2) = 1.9e-84  
Identities = 13/39 (33%), Positives = 16/39 (41%), Frame = +2

Query: 941 SCVAGRLPGLPGGSPSDNPTQARVESGMGGSGFSYQGT 1057  
+ | | | | | | + + | | | |  
Sbjct: 353 TCVEHSHSPGGGGRYSDTPSHPCLCSGTQRSAISSVST 391 (SEQ ID NO:63)

Potassium channels are ubiquitous multisubunit membrane proteins that regulate membrane potential in numerous cell types. One family of mammalian K<sup>+</sup> channels is characterized by the presence of 4 transmembrane domains and 2 P domains per subunit; this family includes TASK, TWIK (KCNK1; OMIM 601745) and TREK (KCNK2; OMIM 603219). See, Duprat *et al.*, 1997 *EMBO J.* 16: 5464-5471. The human cDNA, designated TASK, encodes a 394-amino acid polypeptide with 85% identity to the mouse ortholog. See, Duprat *et al.*, 1997. The sequence contains consensus sites for N-linked glycosylation and for phosphorylation at the C-terminal. Northern blot analysis showed that TASK is expressed in a variety of human tissues, with highest levels in pancreas and placenta. See, Duprat *et al.*, 1997. Expression of the TASK cDNA revealed that the functional protein creates currents that are K(+) -selective, instantaneous, and noninactivating. See, OMIM 603220. These currents showed an outward rectification when external K<sup>+</sup> was low, but evinced absence of activation and inactivation kinetics as well as voltage independence, characteristics of so-called leak or background conductances. See, OMIM 603220. TASK currents were very sensitive to small changes in extracellular pH, suggesting that TASK has a role in cellular responses to changes in extracellular pH. See, OMIM 603220.

Finally, FCTR11 was found to have high homology to the domains shown in Table 11G.

**Table 11G: CD domain analysis of FCTR11**

Sequences producing significant alignments:	Score (bits)	E value
TWIK_channel, TASK K <sup>+</sup> channel	284	5e-78
CNG_membrane, Transmembrane region cyclic Nucleotide Gated Cha...	35.8	0.004

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.



**FCTR12 (AL121574\_A)**

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 12A. A TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in clone RP3-441A12 of chromosome 6.

5 **Table 12A. FCTR12 (AL121574\_A) nucleotide sequence (SEQ ID NO:23).**

natcagactctattgaccgccactctaacggttgtcaggcattgtggcaattgtgtccttgtggctttg  
ggcatttaagcttcactacttgacctctatagttttggcatcttctcatacacatgactatcagcaag  
ctaaattatttactgactgtcctgctccccgcactccgcctttgaggcgcggaacgaagtggcacgcc  
cggatcccagctgatcagcggctgggctttggcggttggtcccccgggcgagaccattgtgactcctc  
10 gggagggggcgacgcggggagggggcgagcgccattgtccggtcagcgcagcctccgggggaggg  
gacggtgttacggagacagcagggcccggggcttcagagcgccgctgcgactccggagccggcgggg  
ggctccggctccttccctgcgccaccgcacaggacatctcttggctggggagcggcggtgagaccgc  
cgagggcgctctgtgtccctcctcccccgcggtcctcgagcggggcccgggccagccgcgcaccgc  
15 tgccgcgcgcgagctccgccgcgcgcgagcaccatgggagacgctgggagcgcgcgcagcaaagcgcc  
cagcctgccgcctcgctgtccctgcggcttctggggactaacggcagttcctttaggattgctgctct  
ttcgagtgacttaggctgcaggacttgctgcccagcattgccagtcaggacactaatcagtgtggct  
cggttgaatag

The encoded C-terminal fragment of the encoded protein is presented using the one-  
20 letter code in Table 12B. The C-terminal fragment disclosed has a very high probability of  
being sorted to the plasma membrane. No cleavage site for a signal peptide was detected.

**Table 12B. Encoded FCTR12 protein sequence (SEQ ID NO:24).**

XQTLTATLTLSGIVAIVSLWLWAFKLHYLTSIVLASSHSDYQQAFLFTDCPAPRTPPLRRGTKWHA  
RIPADQRLGFGVGSFGRDHCDSGGAHAGEGAERPLSGQSLRGRGRGYGDSRARGFRAAAATPEPAG  
25 GSGPSLRHRTGHLGSGWGAAVRPAEGVCVPPPPRSSSGARAQPPPLPPPSSAAAEHHGRRWERAQQSA  
QPAASLSLRLLTNGSSFRIALSSDLGCRTCCPALPSQDTNQC GSVE

In a search of sequence databases, no similarities were found to any known nucleic  
acid or protein.

30 The nucleic acids and proteins of the invention are potentially useful in the treatment  
of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune  
disorders, and hematopoietic disorders.

**FCTR13 (AL121723\_A)**

A novel nucleic acid encoding a novel secreted morphogenic protein is shown in  
35 Table 13A. It was identified in chromosome 20 clone RP5-854E16. An initiation codon is  
shown at the beginning of the sequence and a TGA stop codon was identified at the 3' end  
indicating that this sequence is a coding sequence. These are shown in bold face in Figure  
13A.

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Query: 121 CTACTCCAAGGCTTCAGCCGACTTTTCCTGAAAGATGACCTGCTTCGGGGCATAGACAGC 180  
 |||  
 Sbjct: 225 CTACTCCAAGGCTTCAGCCGACTTTTCCTGAAAGGTAACCTGCTTCGGGGCATAGACAGC 284

5 Query: 181 TTCTTCTCTGCCCCATGGACTTCCGGGGCCTCCCTAGGAACCTACCAACAAGAGGAGAAC 240  
 |||  
 Sbjct: 285 TTATTCTCTGCCCCATGGACTTCCGGGGCCTCCCTGGGAACCTACCACAAAGAGGAGAAC 344

10 Query: 241 GAGGAGCACCAGCTGAGGAACAACACCCTCTCCAGCCACCTCCATATTGACAAGGTGACC 300  
 |||  
 Sbjct: 345 CAGGAGCACCAGCTGGGGAACAACACCCTCTCCAGCCACCTCCAGATCGACAAGATGACC 404

Query: 301 GACAATAAGACAGGAGAGGTGCTGATCTCCGAGAAGGTGGTGGCATCCATCCAGCCGGCG 360  
 |||  
 15 Sbjct: 405 GACAACAAGACAGGAGAGGTGCTGATCTCCGAGAATGTGGTGGCATCCATTCAACCAGCG 464

Query: 361 GAGGGGAGCTTCGAGGGTAAGTGAAGG 388  
 |||  
 20 Sbjct: 465 GAGGGGAGCTTCGAGGGTGATTGAAGG 492 (SEQ ID NO:64)

Score = 1244 (186.7 bits), Expect = 1.6e-123, Sum P(2) = 1.6e-123  
 Identities = 282/322 (87%), Positives = 282/322 (87%), Strand = Plus / Plus

25 Query: 384 GAAGGCGGCGGCCCTGGTGTCATCCGGAAGGCTATGGACAACCTCCATGCAGAGCTCCA 443  
 |||  
 Sbjct: 506 GGAGAAGGAGGCCCTGGTACCCATCCAGAAGGCCACGGACAGCTTCCACACAGAACCTCCA 565

30 Query: 444 TCCCGGGTGGCCTTTTGGATCATGAAGCTGCCACGGTGGAGGTCCCAACCAATGTCCT 503  
 |||  
 Sbjct: 566 TCCCGGGTGGCCTTCTGGATCATTAAAGCTGCCACGGCGGAGGTCCCAACAGGATGCCCT 625

Query: 504 GGAGGGCGGCCCTGGCTCAGTGAGAAGCGACACCGCTGCAGGCCATCCAGGATGGGCT 563  
 |||  
 35 Sbjct: 626 GGAGGGCGGCCACTGGCTCAGCGAGAAGCGACACCGCTGCAGGCCATCCGGGATGGACT 685

Query: 564 CCACGAGGGGACCGCGAGGACGTCTCTAAAAGAGGGGACCCAGGGCTCCTCCCACTCCGG 623  
 |||  
 40 Sbjct: 686 CCGCAAGGGGACCCACAAGGACGTCTCTAGAAGAGGGGACCGAGAGCTCCTCCCACTCCAG 745

Query: 624 GCTGTCTCTCC-GAAAGACCCACTTACTGTACATCTTCAGGCTTTCCTGGCAGCTATAGGG 682  
 |||  
 Sbjct: 746 GCTGTCCCCCGAAAGACCCACTTACTGTACATCCTCAGGCCCTCTCGGCAGCTGTAGGG 805

45 Query: 683 GTTGGGACCGGGGAGCACCTGC 704  
 |||  
 Sbjct: 806 GTGGGACCGGGGAGCACCTGC 827 (SEQ ID NO:65)

Table 13D. BLASTX of FCTR13 with Soggy-1.

>ptnr:TREMBLNEW-ACC:AAF02678 SOGGY-1 PROTEIN - Homo sapiens (Human), 242 aa.  
 Dickkopf-1 (dkk-1) is member of a novel family of secreted proteins and functions  
 in head induction during Xenopus embryogenesis, acting as a potent inhibitor of Wnt  
 signaling.

Score = 813 (286.2 bits), Expect = 6.3e-84, Sum P(2) = 6.3e-84  
 Identities = 166/218 (76%), Positives = 181/218 (83%), Frame = +1

Query: 4 RHPLVLLLLLSALVTSFTAASIHDAAHAQESSLGLTGLQSLQGFSLFLKDDLLRGIDSF 183  
 |||  
 60 Sbjct: 12 RHLLVLLLLLSTLVIPSAAAPIHDADAQESSLGLTGLQSLQGFSLFLKGNLLRGIDSL 71

Query: 184 FSAPMDFRGLPRNYQQEENEHQLRNNLTSSHLHIDKVTDNKTGEVLISEKVVASIQPAE 363  
 |||  
 Sbjct: 72 FSAPMDFRGLPGNYHKEENQEHQLGNNTLSSHLQIDKMTDNKTGEVLISENVVASIQPAE 131

65 Query: 364 GSFEGNWKAA-----ALVSIRKAMDNFHAELHPRVAFWIMKLPWRSHHNVLEGGRWLS 525

Sbjct: 132 GSFEGDLKVPMEKEALVPIQKATDSFHTELHPRVAFWIICKLPRRRSHQDALEGGHWLS 191  
 Query: 526 EKRHLRLQAIQDGLHEGTREDVLKEGTQGSSSHSGLSSER 639  
 Sbjct: 192 EKRHLRLQAIKDGLRKGTGHKDVLEEGTESSSHSRLSPRK 229 (SEQ ID NO:66)

Score = 54 (19.0 bits), Expect = 6.3e-84, Sum P(2) = 6.3e-84  
 Identities = 12/15 (80%), Positives = 12/15 (80%), Frame = +3

Query: 633 RKTHLLYIFRLSWQL 677  
 Sbjct: 228 RKTHLLYILRPSRQL 242 (SEQ ID NO:67)

A multiple sequence alignment for FCTR13 AL121723\_A A is given in Table 13E in a ClustalW analysis comparing the protein of the invention with related protein sequences. The FCTR13 polypeptide is shown on line 1, the human Soggy-1 protein (gi|7657554| ref NP\_055234.1| soggy-1 gene [Homo sapiens])(SEQ ID NO:68) on line 2, mouse Soggy-1 protein (gi|10644567| gb|AAG21340.1| AF274312\_1 (AF274312) soggy precursor [Mus musculus]) (SEQ ID NO:69) on line 3, and mouse Soggy-1 protein (gi|10644569| gb|AAG21341.1| AF274313\_1 (AF274313) soggy precursor [Mus musculus]) (SEQ ID NO:70) on line 4. Table 13E depicts a ClustalW alignment of FCTR13 against proteins from a public database. Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

**Table 13E. ClustalW alignment of the FCTR13.**

10	20	30	40	50	60
FCTR13	-----	MRHPLVLLLLLSALVTS	SFTAAASIHDAFAOESSL	GLTGLQSL	LLQGF
Line 2	-----	MGEASPPAPARRHLLV	LLLLLSTLVTPSAAPI	HDADAQESSL	GLTGLQSL
Line 3	-----	MCRLRVLLLLPLAFV	SSSALPIHDVDSQONT	SGFLGLQRL	LQSF
Line 4	-----	MCRLRVLLLLPLAFV	SSSALPIHDVDSQONT	SGFLGLQRL	LQSF
35	70	80	90	100	110
FCTR13	-----	KDILLRGHDSFFSAP	MDFRGLPRNYQ	QEEENEHQLRNNT	LSSHLHIDK
Line 2	-----	KGNLLRGHDSLFSAP	MDFRGLPCNYHKE	ENQEHQGNNT	LSSHLQIDK
Line 3	-----	KNDLLRDLNFFSSP	MDFRDLPRNEHQ	EENQEHQGNNT	LSSHLQIDK
Line 4	-----	KNDLLRDLNFFSSP	MDFRDLPRNEHQ	EENQEHQGNNT	LSSHLQIDK
40	130	140	150	160	170
FCTR13	-----	EKVVASIQPAEGSF	EGCNWKA-----	ALVSTRKAMDNF	HAELHPRVAFW
Line 2	-----	ENVVASIQPAEGSF	EGDLKVPMEKEAL	VPIQKATDSFHT	ELHPRVAFW
Line 3	-----	EKVEASIEPER-N	PEGDWKVPKVEAK	PPVPVOKVTD	SLHPEPR-QVAF
Line 4	-----	EKVEASIEPER-N	PEGDWK-----	-----	-----
50	190	200	210	220	230
FCTR13	-----	-----	-----	-----	-----
Line 2	-----	-----	-----	-----	-----
Line 3	-----	-----	-----	-----	-----
Line 4	-----	-----	-----	-----	-----
	240				

5	FCTR13	HNVLGGGRWLSEKRHRMQAIDGLHEGTREDVLEKGTQSSSHSGLSSEPTYCTSSGFPG
	Line 2	QDALEGGHWLSEKRHRMQAIDGLRKGTHKDVLEGTSSSHSRISPRK-----
	Line 3	PDVQGGGRWLIKRRHRMQAIDGLRGCAREDSEEGVHIPQHAKLPRK-----
	Line 4	-----
10	FCTR13	SYRGWDRGAPASWVGWVSVSKGLAHGPTGLGLEPGSSGIDFVNAFMEGYSNQINIMVLVQ
	Line 2	-----
	Line 3	-----
	Line 4	-----
15	FCTR13	KRIHRPMQONREARNKATHLPSDLRQS (SEQ ID NO:26)
	Line 2	-----THLLYILRPS--ROL (SEQ ID NO:68)
	Line 3	-----THFLYILRPS--QCL (SEQ ID NO:69)
20	Line 4	----- (SEQ ID NO:70)

From these analyses, it is seen that the FCTR13 AL121723\_A A nucleic acid and protein have a strong similarity with human soggy-1 protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

#### FCTR14 (AL121756\_A)

The novel nucleic acid encoding a novel secreted protein is shown in Table 14A. This sequence contains an initiation codon at the 5' end, and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type. This sequence originates in chromosome 20 clone RP4-726C3.

**Table 14A. FCTR14 (AL121756\_A) nucleotide sequence (SEQ ID NO:27).**

atgctgcggtatcctgtgcctggcactctgcagcctgctgactggcacgcgagctgaccctggggcact  
gctgcggttgggcatggacatcatgaaccgtgaggtccagagcgccatggatgagagtcataatcctgg  
35 agaagatggcagccgagggcaggcaagaacagccagggatgaaacctatcaagggcatcaccaatttg  
aaggtgaaggatgtccagctgcccgctcatcacactgaactttgtacctggagtgggcatcttccaatg  
tgtgtccacaggcatgaccgtcactggcaagagcttcatgggagggaaacatggagatcatcgtggccc  
tgaacatcacagccaccaaccggcttctgcgggatgaggagacaggcctccccgtgttcaagagtga  
ggctgtgaggtcatcctgggtcaatgcagaagactaacctgcctagcaacatgctcccaagatggtcaa  
40 caagtccctggacagcaccctgcacaaagtcctccctgggctgatgtgtcccgcctcgatgcagtcc  
tgggtgatgtgaacaggaagtggaccaacctcagtgaccccatgcctgtggggccagatgggcaccgtc  
aaatatgttctgatgtccgcaccagccaccacagccagctacatccaactggacttcagtcctgtggt  
gcagcagcaaaagggcaaaacatcaagcttgtgatgcggggaggccctcacgttccctgaggggtt  
atgccaaaggctcgtgcgagctgctgctcccagccaccttctctctgcagagcttgccttctgcag  
45 aagtcctttcatgtgaatatccaggatacaatgattgggtgagctgccccacaaaccaccaagacct  
ggctcgcttcatctgaagtggctgtagcttatcccaagtcaaagcccttgacgacccagatcaaga  
taaagaagcctcccaaggctcactatgaagacaggcaagagcctgctgcacctccacagcaccctggag  
atgttcgcagctcggtggcgagcaaggctccaatgtccctcttctcctagaagtgcacttcaatct  
gaaggtccagtaactcagtgcatgagaaccagctgcagatggccacttcttggacagattactgagct  
50 tgtcccgaagtccatcgattggcaacttcaatgagagggaattaactggcttcatcaccagctat

ctcgaagaagcctacatcccagttgtcaatgatgtgcttcaagtggggctcccactcccggactttct  
ggccatgaattacaacctggctgagctggacatagtagagcttgggggcatcatggaacctgccgaca  
tatga

- 5 The encoded protein is presented using the one-letter code in Table 14B. The protein has a moderate probability of being sorted to the plasma membrane. A signal peptide most likely is cleaved between residues 18 and 19, *i.e.*, at the dash in the amino acid sequence TRA-DPG.

**Table 14B.** Encoded FCTR14 protein sequence (SEQ ID NO:28).

10 MLRILCLALCSLLTGTRADPGALLRLGMDIMNREVQSAMDESHILEKMAAEAGKKQPGMKPIKGITNL  
KVKDVQLPVITLNFVPGVGIFQCVSTGMTVTGKSFMGGNMEIIVALNITATNRLLRDEETGLPVFKSE  
GCEVILVNVKTNLPSNMLPKMVNKFLDSTLHKVLPGLMCPAIDAVLVYVNRKWTNLSDPMPVGMGT  
KYVLMSAPATTASYIQLDFSPVVQQQKGTIKLADAGEALTPEGYAKGSSQLLLPATFLSAELALLQ  
15 KSFHVNIQDTMIGELPPQTTLARFIPEVAVAYPKSKPLTTQIKIKKPPKVTMKTGKSLHLHSTLE  
MFAARWRSKAPMSLFLLEVHFNLVQYSVHENQLQMATSLDRLLSLRKSSSIGNFNERELTGFITSY  
LEEAYIPVVNDVLQVGLPLPDFLAMNYNLAELDIVELGIMEPADI

A BLASTN search of sequence databases for the FCTR14 nucleic acid sequence identified significant similarities to the human genomic clone HSDJ726C3, isolated from human DNA sequence from clone RP4-726C3 on chromosome 20. In a BLASTX comparison, it was found that the full FCTR14 amino acid sequence has 130 of 391 residues (33%), are identical to, and 229 of 391 residues (58%) positive with, rat potential ligand-binding protein RY2G5 having a total of 409 amino acid residues (SPTREMBL-ACC:Q05704) (SEQ ID NO:71). The BLASTX alignment is shown in Table 14C.

**Table 14C.** BLASTX alignment of FCTR14

>ptnr:SPTREMBL-ACC:Q05704 POTENTIAL LIGAND-BINDING PROTEIN RY2G5 - RATTUS  
NORVEGICUS (RAT), 470 aa (fragment).  
CC -!- TISSUE SPECIFICITY: SUBREGIONS OF THE OLFACTORY MUCOSA.

30 Score = 579 (203.8 bits), Expect = 2.0e-55, P = 2.0e-55  
Identities = 130/391 (33%), Positives = 229/391 (58%), Frame = +1

Query: 175 MKPIKGITNLKVKDVQLPVITLNFVPGVGIFQCVSTGMTVTGKSFMGGNMEIIVALNITA 354  
+ ++||| ++ ++ || +++ +|||++ + | + + ||| +| ++| | +|||

35 Sbjct: 73 LSTVQGITGLRIVELTLPRVSVRLPGVGVLSLYTRVAINGKSLIGF-LDIAVEVNITA 131

Query: 355 TNRLLRDEETGLPVFKSEGCEVILVNVKTNLPSNMLPKMVNKFLDSTLHKVLPGLMCPAI 534  
|| | || | | + +| +| | +|| +|+ ++ | ||| L+|| +

40 Sbjct: 132 KVRLTMDR-TGYPRLVIERCDTLGGIKVKLLRGLLPNLVDNLVNRVLANVLPDLLCPIV 190

Query: 535 DAVLVYVNRKWTNLSDPMPVGMGTVKYVLMSAPATTASYIQLDFSPVVQQQKGTIKLA 714  
| || || + + +|+| +|+|+| | | | +++| | + +| + | |

Sbjct: 191 DVVLGLVNDQLGLVDSLPLGIILGSVQYTFSSLPVLTGEFLELDLNTLVGEAGGDLDIDYP 250

45 Query: 715 DAGEALT----FPEGYAKG---SSQLLLPATFLSAELALLQK--SFHVNIQDTMIGELPP 867  
| + || | +||| + | |||+ | +||| + ++| | | +|||

Sbjct: 251 LGRPAMLPRPQMPPELPPMGDNTNSQLAISANFLSSVLTMLQKQALDIDITDGMFEDLPP 310

50 Query: 868 QTTKTLARFIPEVAVAYPKSKPLTTQIKIKKPPKVTMKTGKSLHLHSTLEMFARWRSK 1047  
|| || ||+| ||+|+||| +|++ || ||++ |+|+ + +| |+ ++ +

Sbjct: 311 LTTSTLGALIPKVFFQYQYVESRPLTIRIQVNPPTVTLQKDKALVKVFATSEVVVSQ-PND 369

Query: 1048 APMFLFLEVFHFNKQYSVHENQLQMATSLDRLLSLRKSSSIGNFNERELTGFTSYL 1227  
 ++ |++| +| +|| ++| + ||+ ||+ ++|++|||+ | + \*

5 Sbjct: 370 VETTICLIDVDTDLLASFSVEGDKLMIDAKLDKT-SLNLRTSNVGNFDFVLEMLVEKIF 428

Query: 1228 EEAYIPVVNDVLQVGLPLPDFLAMNYNLAELDIVE 1332  
 + |++| +| +| |++| | ++++ |++|++|

10 Sbjct: 429 DLAFMPAMNAILGSGVPLPKILNIDFSNADIDVLE 463

A multiple sequence alignment for FCTR14 AL121756\_A is given in Table 14D, with the protein of the invention being shown on line 3, in a ClustalW analysis comparing the protein of the invention with related protein sequences. Table 14D depicts a ClustalW alignment of FCTR13 with proteins from the public database. The alignment is presented

15 against Q05704 - POTENTIAL LIGAND-BINDING PROTEIN RY2G5 (FRAGMENT) (SEQ ID NO:71) and Q05701 - POTENTIAL LIGAND-BINDING PROTEIN RYA3 (SEQ ID NO:72). Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

TABLE 14D ClustalW alignment of FCTR14 (AL121756\_A) protein

25	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	RLHRRERHPGEHPAGVATGALGPGGGLLGTCG-ITANEGLLAGGGGLGG----- --MMPGVYALLLLWGLATPCGLLETVGTARIDKDEFGKATONSIVGGPILQNVLTGTVT ---MLRRLCLALCSLTGTTRADPGALLRLCMDIMNREYQSANDESHILEK-----
30	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	---GELLGGGGLLGCGVVLGVLEGGLSTVGGITGLRIVELTLPRVSVRLPGVGVL SVNQGLLGAGGLLGCG---GLLSYGGGLFSLVEELSGLKIEELTLPTVSKLLPGVGVL -----MAAEAGKKQPC-----MK-PKKGITNLKVKDVOLEVTINNFVPGVGTQC
35	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	LYTRVAINGKS-LIGFLDAVEVNITAKVRLTMDR-TGYPRLVIERCDTLGGIRVKILR LHTKVSLHSGSPLVGLLOLADEVNVSSKVALGMSP-RCTPILDKRCNTLLG--HISITS VSTGMTVTGKSFMGANMEIIVADNITATNRLRDEETCLPVFKSEGCEVILVNVTNLPS
40	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	GLLENVDNLVNRVLANVLPDLLCPIDVVLGVNDOLGLVDSLVPLGLGSGVQYTFSSL GLLPTPTFGLVETLCKVLPGLLCPVVDVSVVNELLGATLSLVPLGLGSGVEFTLATL NMLPKMVNKFIDSTLHKVLPGLMCPALDAVLVYVNRKWTNUSDPMPVGMGTIVKYVMSA
45	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	PLVTGEFEELDINTVGEAGGDIDYPLCRPAMLRPQMPETPPMGDNTNSOLATISANFL PLISNOYIELDINPIVKSIAQGVLDFFKPR---LP---VKMPPEKEDHT-SCVTVPLYL PATTASYIQLDFSPVYQQQKGTIKLADAG-----EATTFEGYAKGSSQLLPATFL
50	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	SSVLTMLQKQGALDDITDGMFEDLPPLTTSTLGALIPKVFFQYQYVESRPLTIRIQVNPFP NVVFGLLQNGALDDITPEVMVPRNIPPLTTDLAALAPEALGKLEPGQHLLSLRVMKSE SAELALLQKS--FHVNIQDTMGELPPQTTKTLARFTEPEVAVAYEKSRLPTIQIKKKKP
	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	TVTLOKDKALVKVFATSEVVVSQF-NDVETTICLIDVDTDLLASFSVEGDKLMIDAKLDK MILLQNKVTVSTIPVTHVLESVE-QGTVALFQMGVMTLNHLVPSSTTKLHISLSLR KVTMKTGKSLHHTSTLEMFAARWRSKAPMSLFLFLEVFHFNKQYSVHENQLQMATSLDR
	Q05704_RY2G5_RAT Q05701_RYA3_RAT AL121756_A	-TSLNLRTSNVGNFDFVLEMLVEKIFDLAFMPAMNAILGSGVPLPKILNIDFSNADIDV -LVQLASSFSQPFASRLLEWLSDVVRAAYMQKTNHLEVGILPKILNVNFANSVVDV LLSLRKSSSIGNFNERELTGFTSYLEAYIPVVNDVLQVGLPLPDFLAMNYNLAELDI

Q05704\_RY2G5\_RAT    **E** **D** **L** **L** **S** **T** --- (SEQ ID NO: 71)  
 Q05701\_RYA3\_RAT    **E** **I** **N** **A** **V** **T** **V** **P** - (SEQ ID NO: 72)  
 AL121756\_A    **V** **L** **G** **G** **I** **M** **E** **P** **D** **I** (SEQ ID NO: 28)

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Finally, FCTR14 was found to have high homology to the domains shown in Table 14D.

**Table 14D: CD domain analysis of FCTR14**

<b>Sequences producing significant alignments:</b>	<b>Score (bits)</b>	<b>E value</b>
BPI/LBP/CETP C-terminal domain; Bactericidal permeability-incr...	72.8	4e-14
BPI/LBP/CETP N-terminal domain; Bactericidal permeability-incr...	60.8	1e-10
LBP_BPI_CETP, LBP / BPI / CETP family	45.8	5e-06

10        The FCTR14 nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

#### **FCTR14 Nucleic Acids and Polypeptides**

15        One aspect of the invention pertains to isolated nucleic acid molecules that encode FCTR14 polypeptides or biologically-active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify FCTR14-encoding nucleic acids (*e.g.*, FCTR14 mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of FCTR14 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic  
 20        DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

25        An FCTR14 nucleic acid can encode a mature FCTR14 polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein.

30        The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell,



in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated FCTR<sub>X</sub> nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by

recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29 as a hybridization probe, FCTR<sub>X</sub> molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to FCTR<sub>X</sub> nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue.

Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an FCTR<sub>X</sub> polypeptide). A nucleic acid molecule that is

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computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of FCTR<sub>X</sub> polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an FCTR<sub>X</sub> polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms.

Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human FCTR<sub>X</sub> protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, as well as a polypeptide possessing FCTR<sub>X</sub> biological activity. Various biological activities of the FCTR<sub>X</sub> proteins are described below.

An FCTR<sub>X</sub> polypeptide is encoded by the open reading frame ("ORF") of an FCTR<sub>X</sub> nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human FCTR<sub>X</sub> genes allows for the generation of probes and primers designed for use in identifying and/or cloning FCTR<sub>X</sub> homologues in other cell types, *e.g.* from other tissues, as well as FCTR<sub>X</sub>

homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.

Probes based on the human FCTR<sub>X</sub> nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an FCTR<sub>X</sub> protein, such as by measuring a level of an FCTR<sub>X</sub>-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting FCTR<sub>X</sub> mRNA levels or determining whether a genomic FCTR<sub>X</sub> gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an FCTR<sub>X</sub> polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of FCTR<sub>X</sub>" can be prepared by isolating a portion of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, that encodes a polypeptide having an FCTR<sub>X</sub> biological activity (the biological activities of the FCTR<sub>X</sub> proteins are described below), expressing the encoded portion of FCTR<sub>X</sub> protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of FCTR<sub>X</sub>.

## **FCTR<sub>X</sub> Nucleic Acid and Polypeptide Variants**

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, due to degeneracy of the genetic code and thus encode the same FCTR<sub>X</sub> proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

In addition to the human FCTR<sub>X</sub> nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the FCTR<sub>X</sub> polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the FCTR<sub>X</sub> genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an FCTR<sub>X</sub> protein, preferably a vertebrate FCTR<sub>X</sub> protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the FCTR<sub>X</sub> genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the FCTR<sub>X</sub> polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the FCTR<sub>X</sub> polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FCTR<sub>X</sub> proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FCTR<sub>X</sub> cDNAs of the invention can be isolated based on their homology to the human FCTR<sub>X</sub> nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding FCTR<sub>X</sub> proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T<sub>m</sub>) for the specific sequence at a defined ionic strength and pH. The T<sub>m</sub> is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T<sub>m</sub>, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and

100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, e.g., Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND  
 5 EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions  
 10 are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species  
 15 hybridizations). See, e.g., Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

### ***Conservative Mutations***

20 In addition to naturally-occurring allelic variants of FCTR X sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, thereby leading to changes in the amino acid sequences of the encoded FCTR X proteins, without altering the functional ability of said FCTR X proteins. For  
 25 example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the FCTR X proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity.  
 30 For example, amino acid residues that are conserved among the FCTR X proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding FCTR X proteins that contain changes in amino acid residues that are not essential for activity. Such



FCTRX proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

An isolated nucleic acid molecule encoding an FCTRX protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the FCTRX protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an FCTRX coding sequence, such as by

saturation mutagenesis, and the resultant mutants can be screened for FCTR<sub>X</sub> biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant FCTR<sub>X</sub> protein can be assayed for (i) the ability to form protein:protein interactions with other FCTR<sub>X</sub> proteins, other cell-surface proteins, or biologically-active portions thereof, (ii) complex formation between a mutant FCTR<sub>X</sub> protein and an FCTR<sub>X</sub> ligand; or (iii) the ability of a mutant FCTR<sub>X</sub> protein to bind to an intracellular target protein or biologically-active portion thereof; (e.g. avidin proteins).

In yet another embodiment, a mutant FCTR<sub>X</sub> protein can be assayed for the ability to regulate a specific biological function (e.g., regulation of insulin release).

### **Antisense Nucleic Acids**

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FCTR<sub>X</sub> coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an FCTR<sub>X</sub> protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; or antisense nucleic

acids complementary to an FCTR<sub>X</sub> nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an FCTR<sub>X</sub> protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the FCTR<sub>X</sub> protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the FCTR<sub>X</sub> protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of FCTR<sub>X</sub> mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of FCTR<sub>X</sub> mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of FCTR<sub>X</sub> mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine,

pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an FCTR protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site.

Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other. See, *e.g.*, Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, *e.g.*, Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330).

#### **Ribozymes and PNA Moieties**

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These

modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme.

- 5 Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave FCTR<sub>X</sub> mRNA transcripts to thereby inhibit translation of FCTR<sub>X</sub> mRNA. A ribozyme
- 10 having specificity for an FCTR<sub>X</sub>-encoding nucleic acid can be designed based upon the nucleotide sequence of an FCTR<sub>X</sub> cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an FCTR<sub>X</sub>-encoding mRNA.
- 15 See, *e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* FCTR<sub>X</sub> mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, FCTR<sub>X</sub> gene expression can be inhibited by targeting nucleotide

20 sequences complementary to the regulatory region of the FCTR<sub>X</sub> nucleic acid (*e.g.*, the FCTR<sub>X</sub> promoter and/or enhancers) to form triple helical structures that prevent transcription of the FCTR<sub>X</sub> gene in target cells. See, *e.g.*, Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

- In various embodiments, the FCTR<sub>X</sub> nucleic acids can be modified at the base
- 25 moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, *e.g.*, Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is
- 30 replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of FCTR<sub>X</sub> can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of FCTR<sub>X</sub> can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S<sub>I</sub> nucleases (*see*, Hyrup, *et al.*, 1996.*supra*); or as probes or primers for DNA sequence and hybridization (*see*, Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of FCTR<sub>X</sub> can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of FCTR<sub>X</sub> can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, *et al.*, 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon,

1988. *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

### **FCTR<sub>X</sub> Polypeptides**

5 A polypeptide according to the invention includes a polypeptide including the amino acid sequence of FCTR<sub>X</sub> polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, while still  
10 encoding a protein that maintains its FCTR<sub>X</sub> activities and physiological functions, or a functional fragment thereof.

In general, an FCTR<sub>X</sub> variant that preserves FCTR<sub>X</sub>-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or  
15 residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated FCTR<sub>X</sub> proteins, and biologically-  
20 active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FCTR<sub>X</sub> antibodies. In one embodiment, native FCTR<sub>X</sub> proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, FCTR<sub>X</sub> proteins are produced by recombinant DNA techniques.  
25 Alternative to recombinant expression, an FCTR<sub>X</sub> protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FCTR<sub>X</sub> protein is derived, or substantially free from  
30 chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of FCTR<sub>X</sub> proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular

material" includes preparations of FCTR<sub>X</sub> proteins having less than about 30% (by dry weight) of non-FCTR<sub>X</sub> proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FCTR<sub>X</sub> proteins, still more preferably less than about 10% of non-FCTR<sub>X</sub> proteins, and most preferably less than about 5% of non-FCTR<sub>X</sub> proteins. When the FCTR<sub>X</sub> protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the FCTR<sub>X</sub> protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of FCTR<sub>X</sub> proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of FCTR<sub>X</sub> proteins having less than about 30% (by dry weight) of chemical precursors or non-FCTR<sub>X</sub> chemicals, more preferably less than about 20% chemical precursors or non-FCTR<sub>X</sub> chemicals, still more preferably less than about 10% chemical precursors or non-FCTR<sub>X</sub> chemicals, and most preferably less than about 5% chemical precursors or non-FCTR<sub>X</sub> chemicals.

Biologically-active portions of FCTR<sub>X</sub> proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the FCTR<sub>X</sub> proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30) that include fewer amino acids than the full-length FCTR<sub>X</sub> proteins, and exhibit at least one activity of an FCTR<sub>X</sub> protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the FCTR<sub>X</sub> protein. A biologically-active portion of an FCTR<sub>X</sub> protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FCTR<sub>X</sub> protein.

In an embodiment, the FCTR<sub>X</sub> protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. In other embodiments, the FCTR<sub>X</sub> protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in



another embodiment, the FCTR protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and retains the functional activity of the FCTR proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

#### 5 *Determining Homology Between Two or More Sequences*

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

15 The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See*, Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.

25 The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent

sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

### *Chimeric and Fusion Proteins*

The invention also provides FCTR<sub>X</sub> chimeric or fusion proteins. As used herein, an FCTR<sub>X</sub> "chimeric protein" or "fusion protein" comprises an FCTR<sub>X</sub> polypeptide operatively-linked to a non-FCTR<sub>X</sub> polypeptide. An "FCTR<sub>X</sub> polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an FCTR<sub>X</sub> protein (SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30), whereas a "non-FCTR<sub>X</sub> polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FCTR<sub>X</sub> protein, *e.g.*, a protein that is different from the FCTR<sub>X</sub> protein and that is derived from the same or a different organism. Within an FCTR<sub>X</sub> fusion protein the FCTR<sub>X</sub> polypeptide can correspond to all or a portion of an FCTR<sub>X</sub> protein. In one embodiment, an FCTR<sub>X</sub> fusion protein comprises at least one biologically-active portion of an FCTR<sub>X</sub> protein. In another embodiment, an FCTR<sub>X</sub> fusion protein comprises at least two biologically-active portions of an FCTR<sub>X</sub> protein. In yet another embodiment, an FCTR<sub>X</sub> fusion protein comprises at least three biologically-active portions of an FCTR<sub>X</sub> protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the FCTR<sub>X</sub> polypeptide and the non-FCTR<sub>X</sub> polypeptide are fused in-frame with one another. The non-FCTR<sub>X</sub> polypeptide can be fused to the N-terminus or C-terminus of the FCTR<sub>X</sub> polypeptide.

In one embodiment, the fusion protein is a GST-FCTR<sub>X</sub> fusion protein in which the FCTR<sub>X</sub> sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant FCTR<sub>X</sub> polypeptides.

In another embodiment, the fusion protein is an FCTR<sub>X</sub> protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of FCTR<sub>X</sub> can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an FCTR<sub>X</sub>-immunoglobulin fusion protein in which the FCTR<sub>X</sub> sequences are fused to sequences derived from a member of the immunoglobulin protein family. The FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an FCTR<sub>X</sub> ligand and an FCTR<sub>X</sub> protein on the surface of a cell, to thereby suppress FCTR<sub>X</sub>-mediated signal transduction *in vivo*. The FCTR<sub>X</sub>-

immunoglobulin fusion proteins can be used to affect the bioavailability of an FCTR<sub>X</sub> cognate ligand. Inhibition of the FCTR<sub>X</sub> ligand/FCTR<sub>X</sub> interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the

- 5 FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-FCTR<sub>X</sub> antibodies in a subject, to purify FCTR<sub>X</sub> ligands, and in screening assays to identify molecules that inhibit the interaction of FCTR<sub>X</sub> with an FCTR<sub>X</sub> ligand.

- 10 An FCTR<sub>X</sub> chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional
- 15 techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are
- 20 commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An FCTR<sub>X</sub>-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FCTR<sub>X</sub> protein.

#### *FCTR<sub>X</sub> Agonists and Antagonists*

- 25 The invention also pertains to variants of the FCTR<sub>X</sub> proteins that function as either FCTR<sub>X</sub> agonists (*i.e.*, mimetics) or as FCTR<sub>X</sub> antagonists. Variants of the FCTR<sub>X</sub> protein can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the FCTR<sub>X</sub> protein). An agonist of the FCTR<sub>X</sub> protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FCTR<sub>X</sub> protein. An antagonist of the FCTR<sub>X</sub> protein can inhibit one or more of the activities of the naturally occurring form
- 30 of the FCTR<sub>X</sub> protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FCTR<sub>X</sub> protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities

of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the FCTR<sub>X</sub> proteins.

Variants of the FCTR<sub>X</sub> proteins that function as either FCTR<sub>X</sub> agonists (*i.e.*, mimetics) or as FCTR<sub>X</sub> antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the FCTR<sub>X</sub> proteins for FCTR<sub>X</sub> protein agonist or antagonist activity. In one embodiment, a variegated library of FCTR<sub>X</sub> variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FCTR<sub>X</sub> variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential FCTR<sub>X</sub> sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of FCTR<sub>X</sub> sequences therein. There are a variety of methods which can be used to produce libraries of potential FCTR<sub>X</sub> variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FCTR<sub>X</sub> sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.*, Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056; Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

#### *Polypeptide Libraries*

In addition, libraries of fragments of the FCTR<sub>X</sub> protein coding sequences can be used to generate a variegated population of FCTR<sub>X</sub> fragments for screening and subsequent selection of variants of an FCTR<sub>X</sub> protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an FCTR<sub>X</sub> coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S<sub>1</sub> nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the FCTR<sub>X</sub> proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene

products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of FCTR<sub>X</sub> proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FCTR<sub>X</sub> variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

### Anti-FCTR<sub>X</sub> Antibodies

The invention encompasses antibodies and antibody fragments, such as F<sub>ab</sub> or (F<sub>ab</sub>)<sub>2</sub>, that bind immunospecifically to any of the FCTR<sub>X</sub> polypeptides of said invention.

An isolated FCTR<sub>X</sub> protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to FCTR<sub>X</sub> polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length FCTR<sub>X</sub> proteins can be used or, alternatively, the invention provides antigenic peptide fragments of FCTR<sub>X</sub> proteins for use as immunogens. The antigenic FCTR<sub>X</sub> peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and encompasses an epitope of FCTR<sub>X</sub> such that an antibody raised against the peptide forms a specific immune complex with FCTR<sub>X</sub>. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of FCTR<sub>X</sub> that is located on the surface of the protein (*e.g.*, a hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, FCTR<sub>X</sub> protein sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as FCTR<sub>X</sub>. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F<sub>ab</sub> and F<sub>(ab')<sub>2</sub></sub> fragments, and an F<sub>ab</sub> expression library. In a specific embodiment, antibodies to human FCTR<sub>X</sub> proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an FCTR<sub>X</sub> protein sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed FCTR<sub>X</sub> protein or a chemically-synthesized FCTR<sub>X</sub> polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against FCTR<sub>X</sub> can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of FCTR<sub>X</sub>. A monoclonal antibody composition thus typically displays a single binding affinity for a particular FCTR<sub>X</sub> protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular FCTR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to,

the hybridoma technique (*see, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see, e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g.*, Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an FCTR<sub>X</sub> protein (*see, e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F<sub>ab</sub> expression libraries (*see, e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F<sub>ab</sub> fragments with the desired specificity for an FCTR<sub>X</sub> protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotype to an FCTR<sub>X</sub> protein may be produced by techniques known in the art including, but not limited to: (i) an F<sub>(ab')<sub>2</sub></sub> fragment produced by pepsin digestion of an antibody molecule; (ii) an F<sub>ab</sub> fragment generated by reducing the disulfide bridges of an F<sub>(ab')<sub>2</sub></sub> fragment; (iii) an F<sub>ab</sub> fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (iv) F<sub>v</sub> fragments.

Additionally, recombinant anti-FCTR<sub>X</sub> antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449;

Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an FCTR<sub>X</sub> protein is facilitated by generation of hybridomas that bind to the fragment of an FCTR<sub>X</sub> protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an FCTR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-FCTR<sub>X</sub> antibodies may be used in methods known within the art relating to the localization and/or quantitation of an FCTR<sub>X</sub> protein (*e.g.*, for use in measuring levels of the FCTR<sub>X</sub> protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for FCTR<sub>X</sub> proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-FCTR<sub>X</sub> antibody (*e.g.*, monoclonal antibody) can be used to isolate an FCTR<sub>X</sub> polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-FCTR<sub>X</sub> antibody can facilitate the purification of natural FCTR<sub>X</sub> polypeptide from cells and of recombinantly-produced FCTR<sub>X</sub> polypeptide expressed in host cells. Moreover, an anti-FCTR<sub>X</sub> antibody can be used to detect FCTR<sub>X</sub> protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the FCTR<sub>X</sub> protein. Anti-FCTR<sub>X</sub> antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,



5  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### **FCTR<sub>X</sub> Recombinant Expression Vectors and Host Cells**

10 Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an FCTR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

20 Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

30 The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory

sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FCTR proteins, mutant forms of FCTR proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FCTR proteins in prokaryotic or eukaryotic cells. For example, FCTR proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors

include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

5        Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

10        One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.*,  
15        Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

20        In another embodiment, the FCTRX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

25        Alternatively, FCTRX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

30        In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells *see, e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL.

2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to FCTR<sub>X</sub> mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, FCTR<sub>X</sub> protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding FCTR<sub>X</sub> or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) FCTR<sub>X</sub> protein. Accordingly, the invention further provides methods for producing FCTR<sub>X</sub> protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a

recombinant expression vector encoding FCTR<sub>X</sub> protein has been introduced) in a suitable medium such that FCTR<sub>X</sub> protein is produced. In another embodiment, the method further comprises isolating FCTR<sub>X</sub> protein from the medium or the host cell.

### Transgenic FCTR<sub>X</sub> Animals

5           The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FCTR<sub>X</sub> protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous FCTR<sub>X</sub> sequences have been introduced into their genome or homologous

10 recombinant animals in which endogenous FCTR<sub>X</sub> sequences have been altered. Such animals are useful for studying the function and/or activity of FCTR<sub>X</sub> protein and for identifying and/or evaluating modulators of FCTR<sub>X</sub> protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene.

15 Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant

20 animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous FCTR<sub>X</sub> gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FCTR<sub>X</sub>-encoding

25 nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FCTR<sub>X</sub> cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human FCTR<sub>X</sub> gene, such as a mouse FCTR<sub>X</sub>

30 gene, can be isolated based on hybridization to the human FCTR<sub>X</sub> cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the FCTR<sub>X</sub> transgene to

direct expression of FCTR<sub>X</sub> protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the FCTR<sub>X</sub> transgene in its genome and/or expression of FCTR<sub>X</sub> mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding FCTR<sub>X</sub> protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an FCTR<sub>X</sub> gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the FCTR<sub>X</sub> gene. The FCTR<sub>X</sub> gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29), but more preferably, is a non-human homologue of a human FCTR<sub>X</sub> gene. For example, a mouse homologue of human FCTR<sub>X</sub> gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, can be used to construct a homologous recombination vector suitable for altering an endogenous FCTR<sub>X</sub> gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FCTR<sub>X</sub> protein). In the homologous recombination vector, the altered portion of the FCTR<sub>X</sub> gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the FCTR<sub>X</sub> gene to allow for homologous recombination to occur between the exogenous FCTR<sub>X</sub> gene carried by the vector and an endogenous FCTR<sub>X</sub> gene in an embryonic stem cell. The additional flanking FCTR<sub>X</sub> nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is then introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which

the introduced FCTR gene has homologously-recombined with the endogenous FCTR gene are selected. *See, e.g., Li, et al., 1992. Cell 69: 915.*

The selected cells are then injected into a blastocyst of an animal (*e.g., a mouse*) to form aggregation chimeras. *See, e.g., Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152.* A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol. 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.*

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g., Lakso, et al., 1992. Proc. Natl. Acad. Sci. USA 89: 6232-6236.* Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. *See, O'Gorman, et al., 1991. Science 251:1351-1355.* If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.,* by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al., 1997. Nature 385: 810-813.* In brief, a cell (*e.g., a somatic cell*) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.,* through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g., the somatic cell*) is isolated.



## Pharmaceutical Compositions

5 The FCTR<sub>X</sub> nucleic acid molecules, FCTR<sub>X</sub> proteins, and anti-FCTR<sub>X</sub> antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

10 A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

20 Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration,

suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an FCTR<sub>X</sub> protein or anti-FCTR<sub>X</sub> antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar

nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

5           The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.,* U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.,* Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene  
10   therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.,* retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

          The pharmaceutical compositions can be included in a container, pack, or dispenser  
15   together with instructions for administration.

### Screening and Detection Methods

          The isolated nucleic acid molecules of the invention can be used to express FCTR<sub>X</sub> protein (*e.g.,* via a recombinant expression vector in a host cell in gene therapy applications), to detect FCTR<sub>X</sub> mRNA (*e.g.,* in a biological sample) or a genetic lesion in an FCTR<sub>X</sub> gene,  
20   and to modulate FCTR<sub>X</sub> activity, as described further, below. In addition, the FCTR<sub>X</sub> proteins can be used to screen drugs or compounds that modulate the FCTR<sub>X</sub> protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of FCTR<sub>X</sub> protein or production of FCTR<sub>X</sub> protein forms that have decreased or aberrant activity compared to FCTR<sub>X</sub> wild-type protein (*e.g.,* diabetes (regulates insulin  
25   release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-FCTR<sub>X</sub> antibodies of the invention can be used to detect and isolate FCTR<sub>X</sub> proteins and modulate FCTR<sub>X</sub> activity. In yet a further aspect,  
30   the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

          The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

### Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FCTR<sub>X</sub> proteins or have a stimulatory or inhibitory effect on, *e.g.*, FCTR<sub>X</sub> protein expression or FCTR<sub>X</sub> protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an FCTR<sub>X</sub> protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science*

249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of FCTR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an FCTR<sub>X</sub> protein determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FCTR<sub>X</sub> protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FCTR<sub>X</sub> protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>3</sup>H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of FCTR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds FCTR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to FCTR<sub>X</sub> protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of FCTR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FCTR<sub>X</sub> protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FCTR<sub>X</sub> or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with an FCTR<sub>X</sub> target molecule. As used herein, a "target molecule" is a molecule with which an FCTR<sub>X</sub> protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an FCTR<sub>X</sub> interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An FCTR<sub>X</sub> target molecule can be a non-FCTR<sub>X</sub>

molecule or an FCTR<sub>X</sub> protein or polypeptide of the invention . In one embodiment, an FCTR<sub>X</sub> target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound FCTR<sub>X</sub> molecule) through the cell membrane and into the cell. The target,  
 5 for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FCTR<sub>X</sub>.

Determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with an FCTR<sub>X</sub> target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FCTR<sub>X</sub> protein to bind to  
 10 or interact with an FCTR<sub>X</sub> target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an FCTR<sub>X</sub>-responsive  
 15 regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an FCTR<sub>X</sub> protein or biologically-active portion thereof with a test compound and  
 20 determining the ability of the test compound to bind to the FCTR<sub>X</sub> protein or biologically-active portion thereof. Binding of the test compound to the FCTR<sub>X</sub> protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the FCTR<sub>X</sub> protein or biologically-active portion thereof with a known compound which binds FCTR<sub>X</sub> to form an assay mixture, contacting the assay  
 25 mixture with a test compound, and determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to FCTR<sub>X</sub> or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting  
 30 FCTR<sub>X</sub> protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the FCTR<sub>X</sub> protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FCTR<sub>X</sub> can be accomplished, for example, by determining the ability of the FCTR<sub>X</sub> protein to bind to an FCTR<sub>X</sub> target molecule by one of

the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FCTR<sub>X</sub> protein can be accomplished by determining the ability of the FCTR<sub>X</sub> protein further modulate an FCTR<sub>X</sub> target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the FCTR<sub>X</sub> protein or biologically-active portion thereof with a known compound which binds FCTR<sub>X</sub> protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein comprises determining the ability of the FCTR<sub>X</sub> protein to preferentially bind to or modulate the activity of an FCTR<sub>X</sub> target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of FCTR<sub>X</sub> protein. In the case of cell-free assays comprising the membrane-bound form of FCTR<sub>X</sub> protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of FCTR<sub>X</sub> protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either FCTR<sub>X</sub> protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FCTR<sub>X</sub> protein, or interaction of FCTR<sub>X</sub> protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FCTR<sub>X</sub> fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or FCTR<sub>X</sub>



protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of FCTR<sub>X</sub> protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the FCTR<sub>X</sub> protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FCTR<sub>X</sub> protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FCTR<sub>X</sub> protein or target molecules, but which do not interfere with binding of the FCTR<sub>X</sub> protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FCTR<sub>X</sub> protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FCTR<sub>X</sub> protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FCTR<sub>X</sub> protein or target molecule.

In another embodiment, modulators of FCTR<sub>X</sub> protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FCTR<sub>X</sub> mRNA or protein in the cell is determined. The level of expression of FCTR<sub>X</sub> mRNA or protein in the presence of the candidate compound is compared to the level of expression of FCTR<sub>X</sub> mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FCTR<sub>X</sub> mRNA or protein expression based upon this comparison. For example, when expression of FCTR<sub>X</sub> mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FCTR<sub>X</sub> mRNA or protein expression. Alternatively, when expression of FCTR<sub>X</sub> mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FCTR<sub>X</sub> mRNA or protein expression. The level of FCTR<sub>X</sub> mRNA or protein expression in the cells can be determined by methods described herein for detecting FCTR<sub>X</sub> mRNA or protein.

In yet another aspect of the invention, the FCTR<sub>X</sub> proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993.

5 *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with FCTR<sub>X</sub> ("FCTR<sub>X</sub>-binding proteins" or "FCTR<sub>X</sub>-bp") and modulate FCTR<sub>X</sub> activity. Such FCTR<sub>X</sub>-binding proteins are also likely to be involved in the propagation of signals by the FCTR<sub>X</sub> proteins as, for example, upstream or downstream elements of the FCTR<sub>X</sub> pathway.

10 The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FCTR<sub>X</sub> is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an  
15 unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an FCTR<sub>X</sub>-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional  
20 regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FCTR<sub>X</sub>.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

## 25 ***Detection Assays***

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with  
30 genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

## Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the FCTR<sub>X</sub> sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments or derivatives thereof, can be used to map the location of the FCTR<sub>X</sub> genes, respectively, on a chromosome. The mapping of the FCTR<sub>X</sub> sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, FCTR<sub>X</sub> genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the FCTR<sub>X</sub> sequences. Computer analysis of the FCTR<sub>X</sub> sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the FCTR<sub>X</sub> sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g., D'Eustachio, et al., 1983. Science 220: 919-924.* Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the FCTR<sub>X</sub> sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one

step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually.

- 5 The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see*, Verma, *et al.*, HUMAN CHROMOSOMES:  
10 A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more  
15 likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line  
20 through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, **325**: 783-787.

Moreover, differences in the DNA sequences between individuals affected and  
25 unaffected with a disease associated with the FCTR gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome  
30 spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### Tissue Typing

The FCTR<sub>X</sub> sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FCTR<sub>X</sub> sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The FCTR<sub>X</sub> sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

### Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly,

one aspect of the invention relates to diagnostic assays for determining FCTR<sub>X</sub> protein and/or nucleic acid expression as well as FCTR<sub>X</sub> activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant FCTR<sub>X</sub> expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with FCTR<sub>X</sub> protein, nucleic acid expression or activity. For example, mutations in an FCTR<sub>X</sub> gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with FCTR<sub>X</sub> protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining FCTR<sub>X</sub> protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTR<sub>X</sub> in clinical trials.

These and other agents are described in further detail in the following sections.

#### *Diagnostic Assays*

An exemplary method for detecting the presence or absence of FCTR<sub>X</sub> in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes FCTR<sub>X</sub> protein such that the presence of FCTR<sub>X</sub> is detected in the biological sample. An agent for detecting FCTR<sub>X</sub> mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to FCTR<sub>X</sub> mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FCTR<sub>X</sub> nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23,

25, 27, and 29, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to FCTR<sub>X</sub> mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

5           An agent for detecting FCTR<sub>X</sub> protein is an antibody capable of binding to FCTR<sub>X</sub> protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a  
10 detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with  
15 fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect FCTR<sub>X</sub> mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of FCTR<sub>X</sub> mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of FCTR<sub>X</sub> protein  
20 include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of FCTR<sub>X</sub> genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of FCTR<sub>X</sub> protein include introducing into a subject a labeled anti-FCTR<sub>X</sub> antibody. For example, the antibody can be labeled with a radioactive marker whose  
25 presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

30           In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting FCTR<sub>X</sub> protein, mRNA, or genomic DNA, such that the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA is detected in the biological sample, and

comparing the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA in the control sample with the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of FCTR<sub>X</sub> in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting FCTR<sub>X</sub> protein or mRNA in a biological sample; means for determining the amount of FCTR<sub>X</sub> in the sample; and means for comparing the amount of FCTR<sub>X</sub> in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect FCTR<sub>X</sub> protein or nucleic acid.

#### *Prognostic Assays*

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with FCTR<sub>X</sub> protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity in which a test sample is obtained from a subject and FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FCTR<sub>X</sub> expression or activity in which a test sample is obtained and FCTR<sub>X</sub> protein or nucleic acid is detected (*e.g.*, wherein the presence of FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject that can be



administered the agent to treat a disorder associated with aberrant FCTR<sub>X</sub> expression or activity).

The methods of the invention can also be used to detect genetic lesions in an FCTR<sub>X</sub> gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an FCTR<sub>X</sub>-protein, or the misexpression of the FCTR<sub>X</sub> gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from an FCTR<sub>X</sub> gene; (ii) an addition of one or more nucleotides to an FCTR<sub>X</sub> gene; (iii) a substitution of one or more nucleotides of an FCTR<sub>X</sub> gene, (iv) a chromosomal rearrangement of an FCTR<sub>X</sub> gene; (v) an alteration in the level of a messenger RNA transcript of an FCTR<sub>X</sub> gene, (vi) aberrant modification of an FCTR<sub>X</sub> gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an FCTR<sub>X</sub> gene, (viii) a non-wild-type level of an FCTR<sub>X</sub> protein, (ix) allelic loss of an FCTR<sub>X</sub> gene, and (x) inappropriate post-translational modification of an FCTR<sub>X</sub> protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an FCTR<sub>X</sub> gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the FCTR<sub>X</sub>-gene (*see*, Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an FCTR<sub>X</sub> gene under conditions such that hybridization and amplification of the FCTR<sub>X</sub> gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is

anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional  
 5 amplification system (*see*, Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Q $\beta$  Replicase (*see*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

10 In an alternative embodiment, mutations in an FC $\beta$ TRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates  
 15 mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in FC $\beta$ TRX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing  
 20 hundreds or thousands of oligonucleotides probes. *See, e.g.*, Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in FC $\beta$ TRX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify  
 25 base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the  
 30 other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FC $\beta$ TRX gene and detect mutations by comparing the sequence of the sample FC $\beta$ TRX with the corresponding wild-type (control) sequence.

Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (*see, e.g.,* PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the FCTR<sub>X</sub> gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.*, 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FCTR<sub>X</sub> sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S<sub>1</sub> nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,* Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FCTR<sub>X</sub> cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g.,* Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an FCTR<sub>X</sub> sequence, *e.g.,* a wild-type FCTR<sub>X</sub> sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.,* U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FCTRX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g., Orita, et al., 1989. Proc. Natl. Acad. Sci. USA: 86: 2766; Cotton,*

5 Single-stranded DNA fragments of sample and control FCTRX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with

10 labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

15 In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature 313: 495.* When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting

20 GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer

25 extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature 324: 163; Saiki, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 6230.* Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the

30 oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the

molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.,* Gasparini, *et al.*, 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.,* Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an FCTR<sub>X</sub> gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which FCTR<sub>X</sub> is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

#### Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on FCTR<sub>X</sub> activity (*e.g.,* FCTR<sub>X</sub> gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.,* the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.,* drugs) for prophylactic or therapeutic treatments based on a

consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of FCTR protein, expression of FCTR nucleic acid, or mutation content of FCTR genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of FCTR<sub>X</sub> protein, expression of FCTR<sub>X</sub> nucleic acid, or mutation content of FCTR<sub>X</sub> genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an FCTR<sub>X</sub> modulator, such as a modulator identified by one of the exemplary screening assays described herein.

#### *Monitoring of Effects During Clinical Trials*

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTR<sub>X</sub> (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FCTR<sub>X</sub> gene expression, protein levels, or upregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting decreased FCTR<sub>X</sub> gene expression, protein levels, or downregulated FCTR<sub>X</sub> activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FCTR<sub>X</sub> gene expression, protein levels, or downregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting increased FCTR<sub>X</sub> gene expression, protein levels, or upregulated FCTR<sub>X</sub> activity. In such clinical trials, the expression or activity of FCTR<sub>X</sub> and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including FCTR<sub>X</sub>, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates FCTR<sub>X</sub> activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of FCTR<sub>X</sub> and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of FCTR<sub>X</sub> or other genes. In this manner, the gene expression pattern can serve as a marker, indicative

of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the pre-administration sample with the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of FCTR<sub>X</sub> to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of FCTR<sub>X</sub> to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

### Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FCTR<sub>X</sub> expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. These methods of treatment will be discussed more fully, below.

#### *Disease and Disorders*

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs,



derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

#### *Prophylactic Methods*

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant FCTR<sub>X</sub> expression or activity, by administering to the subject an agent that modulates FCTR<sub>X</sub> expression or at least one FCTR<sub>X</sub> activity. Subjects at risk for a disease that is caused or contributed to by aberrant FCTR<sub>X</sub> expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FCTR<sub>X</sub> aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of FCTR<sub>X</sub> aberrancy, for example, an FCTR<sub>X</sub> agonist or FCTR<sub>X</sub> antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening

assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

### *Therapeutic Methods*

Another aspect of the invention pertains to methods of modulating FCTR<sub>X</sub> expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of FCTR<sub>X</sub> protein activity associated with the cell. An agent that modulates FCTR<sub>X</sub> protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an FCTR<sub>X</sub> protein, a peptide, an FCTR<sub>X</sub> peptidomimetic, or other small molecule.

In one embodiment, the agent stimulates one or more FCTR<sub>X</sub> protein activity. Examples of such stimulatory agents include active FCTR<sub>X</sub> protein and a nucleic acid molecule encoding FCTR<sub>X</sub> that has been introduced into the cell. In another embodiment, the agent inhibits one or more FCTR<sub>X</sub> protein activity. Examples of such inhibitory agents include antisense FCTR<sub>X</sub> nucleic acid molecules and anti-FCTR<sub>X</sub> antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an FCTR<sub>X</sub> protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) FCTR<sub>X</sub> expression or activity. In another embodiment, the method involves administering an FCTR<sub>X</sub> protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FCTR<sub>X</sub> expression or activity.

Stimulation of FCTR<sub>X</sub> activity is desirable in situations in which FCTR<sub>X</sub> is abnormally downregulated and/or in which increased FCTR<sub>X</sub> activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preeclampsia).

### **Determination of the Biological Effect of the Therapeutic**

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

### **Prophylactic and Therapeutic Uses of the Compositions of the Invention**

The FCTR<sub>X</sub> nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the FCTR<sub>X</sub> protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the FCTR<sub>X</sub> protein, and the FCTR<sub>X</sub> protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

### **EXAMPLES**

The following examples illustrate by way of non-limiting example various aspects of the invention.

### Example 1: Method of Identifying the Nucleic Acids

The novel nucleic acids of the invention were identified by TblastN using CuraGen Corporation's sequence file, run against the Genomic Daily Files made available by GenBank. The nucleic acids were further predicted by the program GenScan™, including  
 5 selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length proteins.

### Example 2. Quantitative expression analysis of FCTR2 in various cells and tissues

The quantitative expression of clone AL078594\_A (FCTR2) was assessed in a large  
 10 number of normal and tumor sample cells and cell lines (Panel 1), as well as in surgical tissue samples (Panel 2), by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

First, 96 RNA samples were normalized to  $\beta$ -actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription  
 15 Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48°C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using  $\beta$ -actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TAQMAN® universal  
 20 PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with  
 25 the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for  $\beta$ -actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their  $\beta$ -actin  
 30 /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-

specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature ( $T_m$ ) range = 58°-60° C, primer optimal  $T_m$  = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe  $T_m$  must be 10° C greater than primer  $T_m$ , amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthesgen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

The expression was probed with the primer-probe set Ag 259. The Forward primer sequence is 5'-GGAGAGGCTCTGAAGCTACACAA-3' (SEQ ID NO:31); the Probe primer sequence is TET-5'-TCAGCTGCACAAGCCCCCTGCT-3'-TAMRA (SEQ ID NO:32); and the Reverse primer sequence is 5'-GCAGTGGTTGGAGCTGGAA-3' (SEQ ID NO:33). Table 15 shows the primer locations within the FCTR2 nucleic acid sequence.

Table 15. Primer-Probe Set Ag259

Primers	Length	Start Position
Forward	23	124
Probe	22	158
Reverse	19	181

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl<sub>2</sub>, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

The results for various cells and cell lines that constitute Panel 1 are shown in Table 16. In Table 16, the following abbreviations are used: ca. = carcinoma; \* = established from metastasis; met = metastasis; s cell var= small cell variant; non-s = non-sm =non-small;

squam = squamous; pl. eff = pl effusion = pleural effusion; glio = glioma; astro = astrocytoma; and neuro = neuroblastoma.

Table 16.

Tissue Name	Rel. Expr., %	Tissue Name	Rel. Expr., %
Adipose	100.0	Colon ca. HT29	0.2
Adrenal gland	0.0	Colon ca. CaCo-2	0.0
Bladder	0.2	Colon ca. HCT-15	3.0
Bone marrow	0.0	Colon ca. HCT-116	0.0
Endothelial cells	0.0	Colon ca. HCC-2998	0.2
Endothelial cells (treated)	0.0	Colon ca. SW480	1.5
Liver	1.5	Colon ca.* (SW480 met)SW620	0.0
Liver (fetal)	0.0	Fetal Skeletal	0.3
Spleen	0.0	Skeletal muscle	2.6
Thymus	0.0	Heart	6.4
Thyroid	0.0	Stomach	0.0
Trachea	0.0	Gastric ca.* (liver met) NCI-N87	0.3
Testis	0.1	Kidney	4.0
Spinal cord	0.6	Kidney (fetal)	0.1
Salivary gland	0.0	Renal ca. 786-0	0.0
Brain (amygdala)	0.0	Renal ca. A498	0.1
Brain (cerebellum)	2.9	Renal ca. ACHN	0.0
Brain (hippocampus)	0.0	Renal ca. TK-10	0.1
Brain (substantia nigra)	4.8	Renal ca. UO-31	0.1
Brain (thalamus)	0.1	Renal ca. RXF 393	0.0
Cerebral Cortex	0.0	Pancreas	1.5
Brain (whole)	0.0	Pancreatic ca. CAPAN 2	0.2
Brain (fetal)	0.0	Ovary	0.2
CNS ca. (glio/astro) U-118-MG	0.2	Ovarian ca. IGROV-1	0.7
CNS ca. (astro) SF-539	0.0	Ovarian ca. OVCAR-3	51.1
CNS ca. (astro) SNB-75	0.0	Ovarian ca. OVCAR-4	52.9
CNS ca. (astro) SW1783	0.0	Ovarian ca. OVCAR-5	21.6
CNS ca. (glio) U251	0.2	Ovarian ca. OVCAR-8	0.2
CNS ca. (glio) SF-295	0.0	Ovarian ca.* (ascites) SK-OV-3	0.0
CNS ca. (glio) SNB-19	3.3	Prostate	0.0
CNS ca. (glio/astro) U87-MG	0.0	Prostate ca.* (bone met)PC-3	0.0
CNS ca.* (neuro; met ) SK-N-AS	0.0	Placenta	0.0
Small intestine	0.1	Pituitary gland	0.5
Colorectal	0.1	Uterus	0.0

It is seen from Table 16 that there is high expression of sequence AL078594\_A found in several ovarian cancer cell lines, and very high expression in normal adipose tissue.

## **Panel 2**

Panel 2 consists of a 96 well plate (2 control wells, 94 test samples) composed of RNA/cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues procured are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins". The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table 17). In addition, RNA/cDNA was obtained from various human tissues derived from human autopsies performed on deceased elderly people or sudden death victims (accidents, etc.). These tissue were ascertained to be free of disease and were purchased from various high quality commercial sources such as Clontech, Research Genetics, and Invitrogen.

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electrophoresis using 28s and 18s ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the presence of low molecular weight RNAs indicative of degradation products. Samples are quality controlled for genomic DNA contamination by reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

**Table 17.**

<b>Tissue Name</b>	<b>Rel. Expr. %</b>	<b>Tissue Name</b>	<b>Rel. Expr. %</b>
Normal Colon GENPAK 061003	0.0	Kidney Cancer Clontech 8120607	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	Kidney NAT Clontech 8120608	0.0
83220 CC NAT (ODO3866)	0.0	Kidney Cancer Clontech 8120613	0.0
83221 CC Gr.2 rectosigmoid	0.0	Kidney NAT Clontech 8120614	0.0

(ODO3868)			
83222 CC NAT (ODO3868)	0.0	Kidney Cancer Clontech 9010320	0.0
83235 CC Mod Diff (ODO3920)	0.0	Kidney NAT Clontech 9010321	0.0
83236 CC NAT (ODO3920)	0.0	Normal Uterus GENPAK 061018	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	Uterus Cancer GENPAK 064011	0.0
83238 CC NAT (ODO3921)	0.0	Normal Thyroid Clontech A+ 6570-1**	0.0
83241 CC from Partial Hepatectomy (ODO4309)	0.0	Thyroid Cancer GENPAK 064010	0.0
83242 Liver NAT (ODO4309)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Thyroid NAT INVITROGEN A302153	0.0
87473 Lung NAT (OD04451-02)	0.0	Normal Breast GENPAK 061019	0.0
Normal Prostate Clontech A+ 6546-1	0.0	84877 Breast Cancer (OD04566)	0.0
84140 Prostate Cancer (OD04410)	0.0	85975 Breast Cancer (OD04590-01)	0.0
84141 Prostate NAT (OD04410)	0.0	85976 Breast Cancer Mets (OD04590-03)	0.0
87073 Prostate Cancer (OD04720-01)	0.0	87070 Breast Cancer Metastasis (OD04655-05)	0.0
87074 Prostate NAT (OD04720-02)	0.0	GENPAK Breast Cancer 064006	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	34.6
83239 Lung Met to Muscle (ODO4286)	0.0	Breast NAT Clontech 9100265	100.0
83240 Muscle NAT (ODO4286)	0.0	Breast Cancer INVITROGEN A209073	0.0
84136 Lung Malignant Cancer (OD03126)	0.1	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	0.0	Normal Liver GENPAK 061009	0.0
84871 Lung Cancer (OD04404)	0.0	Liver Cancer GENPAK 064003	0.0
84872 Lung NAT (OD04404)	0.0	Liver Cancer Research Genetics RNA 1025	0.0
84875 Lung Cancer (OD04565)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
85950 Lung Cancer (OD04237-01)	0.2	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0
85970 Lung NAT (OD04237-02)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83256 Liver NAT (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung	0.0	Normal Bladder GENPAK 061001	0.0



(OD04321)			
84138 Lung NAT (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	0.3
Normal Kidney GENPAK 061008	0.0	Bladder Cancer INVITROGEN A302173	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	87071 Bladder Cancer (OD04718-01)	0.0
83787 Kidney NAT (OD04338)	0.0	87072 Bladder Normal Adjacent (OD04718-03)	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Normal Ovary Res. Gen.	0.0
83789 Kidney NAT (OD04339)	0.0	Ovarian Cancer GENPAK 064008	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	87492 Ovary Cancer (OD04768-07)	0.0
83791 Kidney NAT (OD04340)	0.0	87493 Ovary NAT (OD04768-08)	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Normal Stomach GENPAK 061017	0.0
83793 Kidney NAT (OD04348)	0.0	NAT Stomach Clontech 9060359	0.0
87474 Kidney Cancer (OD04622-01)	0.0	Gastric Cancer Clontech 9060395	0.0
87475 Kidney NAT (OD04622-03)	0.0	NAT Stomach Clontech 9060394	0.0
85973 Kidney Cancer (OD04450-01)	0.0	Gastric Cancer Clontech 9060397	0.0
85974 Kidney NAT (OD04450-03)	0.0	NAT Stomach Clontech 9060396	0.5
		Gastric Cancer GENPAK 064005	0.2

There is high expression of sequence AL078594\_A found in normal adjacent breast tissue and in breast cancer tissue. Panel 2 includes only two ovarian cancer samples, neither of which express this sequence.

- 5 Therefore, the FCTR2 protein of clone AL078594\_A may serve as the target for a diagnostic assay in certain ovarian cancers, and as a potential therapeutic target for this subset of ovarian cancer and possibly for breast cancer.

The citation of any reference herein should not be deemed as an admission that such reference is available as prior art to the instant invention.

10

## EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may

be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and  
5 modifications considered to be within the scope of the following claims.

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